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The Future of Physiology is Today

The Korean Journal of
Physiology &
Pharmacology

FAOPS
2023

Nov 1WED–4SAT, EXCO, DAEGU, KOREA
The 10th Federation of the Asian and Oceanian Physiological Societies Congress

in conjunction with the 75th Annual Meeting of the Korean Physiological Society

Abstract Book

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Aims and Scope

The Korean Journal of Physiology & Pharmacology (Korean J. Physiol. Pharmacol., KJPP) is the official journal of both the Korean Physiological Society (KPS) and the Korean Society of Pharmacology (KSP). The journal launched in 1997 and is published bi-monthly in English. KJPP publishes original, peer-reviewed, scientific research-based articles that report successful advances in physiology and pharmacology. KJPP welcomes the submission of all original research articles in the field of physiology and pharmacology, especially the new and innovative findings. The scope of researches includes the action mechanism, pharmacological effect, utilization, and interaction of chemicals with biological system as well as the development of new drug targets. Theoretical articles that use computational models for further understanding of the physiological or pharmacological processes are also welcomed. Investigative translational research articles on human disease with an emphasis on physiology or pharmacology are also invited. KJPP does not publish work on the actions of crude biological extracts of either unknown chemical composition (e.g. unpurified and unvalidated) or unknown concentration. Reviews are normally commissioned, but consideration will be given to unsolicited contributions. All papers accepted for publication in KJPP will appear simultaneously in the printed Journal and online.

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Welcome Message

The President of the The 10th Federation of the Asian and Oceanian Physiological Societies Congress

On behalf of FAOPS and the Korean Physiological Society, I would like to extend a warm welcome to the participants of the 10th Federation of the Asian and Oceanian Physiological Societies Congress (FAOPS 2023), which will be held in Daegu, Korea from November 1–4, 2023.

We have prepared 16 celebration lecture/plenary lectures/special lectures, 54 symposia, and various cultural and sightseeing programs for the 4 days of FAOPS 2023.

The organizers of FAOPS 2023, including the chairman of the organizing committee, Prof. Jin Han, put in a lot of effort to make FAOPS 2023 a success. Nevertheless, I am concerned that there is still something lacking in the treatment of each participant.

I would like to ask for the generous understanding of the participants, even if there are some shortcomings, and hope that you will enjoy the four days of academic feast, Korean culture, and sightseeing in Daegu to your heart's content, so that you can capture the joy of physiology and the beauty of Korea in your heart.

Chae-Hun Leem, MD, PhD
President, FAOPS/The Korean Society of Physiology

The Chair of the FAOPS 2023 Organizing Committee

Dear colleagues and friends,

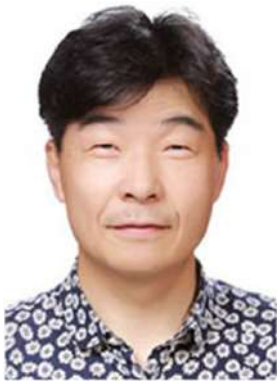
The FAOPS 2023 Congress will be held on November 1–4, 2023 in Daegu, Korea. All members of the Organizing Committee and I are pleased to invite you to one of the largest international gatherings in the field of physiology.

The congress program will provide the exciting education and engaging networking opportunities that we have all missed over the past few years. In addition to abstract presentations and the inspiring talks by world-renowned speakers, there will be a variety of interactive sessions and networking options. The FAOPS Congress is an excellent opportunity to initiate or enhance ongoing collaboration and knowledge.

Located in the south of the Korean Peninsula and with more than 2.4 million population, Daegu is close to 13 of South Korea's official World Heritage Sites of UNESCO. Daegu offers the most authentic Korean culture and is a fascinating destination for all participants.

With so much to see and do, including a variety of spaces and works of art that blend tradition and modernity, we hope you have the best time in Daegu.

We look forward to seeing you in Daegu in November 2023.



Jin Han, MD, PhD
Chair, on behalf of FAOPS 2023 organizing committee

Day 1 November 1, Wed. Program at a Glance

KST (Korea Standard Time)	Room 325	Room 324	Room 323	Room 322	Room 321	Room 320	Room 306 (A)	Grand Ballroom (A), 3F Lobby
09:00 –	Registration (Lobby, 3F)							
10:00 – 11:00			Poster Oral Presentation	Poster Oral Presentation	Poster Oral Presentation	FAOPS Teaching Workshop 2; How to choose "Must Know" lecture topics in medical physiology	FAOPS Teaching Workshop 1; Constructing inclusive student centered learning sessions (ISCLS) in Physiology: through the lens of Universal Design	Poster Viewing & Exhibition
11:00 – 12:00		Present (2019–2023) Council Meeting Time: 10:30–13:00						
12:00 – 13:00								
13:00 – 13:20	**Opening Ceremony** Venue: Room 325							
13:20 – 13:40	**CL 1** **Denis Noble** The future of physiology is today S5							
13:40 – 14:00	**CL 2** **Martin Morad** Cardiac EC-coupling garden with Asian friends: the fruits we picked, much remains! S5							
14:00 – 15:00	**PL 1** **Ronald Evans** Physiology and its transcriptional underpinning S5							
15:00 – 15:10	Break							
15:10 – 17:10	**S1** Computational physiology of ion channels S10	**S2** New physiological insights into how muscle contraction creates new normal mitochondria S11	**S3** Mechanism and new therapeutic strategy for cardiac arrhythmias S12	**S4** RNA modifications in the brain S13	**S5** Taste: Integrating chemical signals from tongue, gut and brain S14	**S6** Occupational health issues among workers of informal sectors: A physiological perspective S15	**S55** Advanced technology for cardiovascular diseases S17	
17:10 – 17:30								
17:30 – 20:00	**Welcome Reception** Venue: Gran Patio Hall, Hotel Inter-Burgo EXCO							

Day 2 November 2, Thu. Program at a Glance

KST (Korea Standard Time)	Room 325	Room 324	Room 323	Room 322	Room 321	Room 320	Room 306 (A)	Room 306 (B)	Grand Ballroom (A), 3F Lobby
08:00 –	Registration (Lobby, 3F)								
09:00 – 11:00	**S7** Physiological functions of glial cell for brain functions S19	**S8** Trends and future of cardiovascular medicine S20	**S9** Beyond the pandemic: Evaluating and aiding the development of students' skills in laboratory classes S22	**S10** Physiological basis of human aging: Implication of lifestyle factors S23	**S11** Behaviors and circuits of model animals S24	**S12** Regulation of cardiovascular and skeletal muscle function in exercise S26	**S40** Metabolic disease and signaling S27	KPS Board Meeting	Poster Viewing & Exhibition
11:00 – 11:10	Break								
11:10 – 12:00	**SL 1** **Nabekura Junichi** From passive "glue" to dynamic renovators: Active surveillance and remodelling of neuronal circuits by microglia and astrocytes S6	**SL 2** **Mario Delmar** Molecular mechanisms of sudden cardiac arrest in young athletes: The case of ARVC S6	**SL 3** **Gou Young Koh** Exploring novel lymphatics for brain clearance S7						
12:00 – 13:20	Luncheon Seminar 1 (Scitech Korea Inc.)	Luncheon Seminar 2 (BIOENGINE Inc.)	Luncheon Seminar 3 (National Research Foundation of Korea)						
13:20 – 15:20	**S13** Novel mechanism and therapeutic strategy of cardiometabolic disease S29	**S14** Pathophysiology of brain diseases: Preclinical mouse model studies S30	**S15** Mitochondria and stress S31	**S16** Concepts of nutraceuticals-induced autophagy for longevity S32	**S17** Tumor microenvironment and plasticity S34	**S24** Educational symposium 2: New technology in physiology teaching: with panel discussion S35	**S56** Frontiers in live cell and intravital imaging technology S36	FAOPS General Assembly	
15:20 – 15:30	Break								
15:30 – 16:20	**SL 4** **Shaw-Jenq Tsai** Pathophysiological functions of dual specificity phosphatase-2 in human diseases S7	**SL 5** **Robyn Murphy** Cellular specific abundance of dysferlin in skeletal muscle S7	**SL 6** **Uhtaek Oh** Tentonin 3, a novel mechanosensitive channel with slow inactivation kinetics: Its biophysical property and physiological functions S7	**Special Education Lecture** **Noriyuki Koibuchi** Educating humanities in physiology education S8					
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16:30 – 18:30	**S19** Symbiosis and dysbiosis of microbiota in relation to human health (Microbiome) S37	**S20** New insight on the role of neuroplasticity: Strategy in management of neurodegenerative diseases S38	**S21** Recent achievement in the excitation-contraction coupling of skeletal muscle S40	**S22** Structure-function relationship of membrane ion channels with atomic resolution S41	**S23** Anatomy and physiology of neural circuits S42	**S18** Educational symposium 1: Collaborative approach of basic-clinical teachers in teaching: panel discussion S43	**S39** Non-motor physiological function of the cerebellum S45		

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08:00 –	Registration (Lobby, 3F)								
09:00 – 10:00	**PL 2** **Hee-Sup Shin** Neural mechanism of affective empathy S5								Poster Viewing & Exhibition
10:00 – 10:10	**Break**								
10:10 – 12:10	**S25** Cellular senescence in metabolic diseases: A therapeutic target S47	**S26** Controlling the tumor microenvironment S48	**S27** The vagus nerve: Normal physiological control and role in pathophysiology S49	**S28** A new vista of physiological mechanisms of chronic pain S50	**S52** Neuro-glia-vascular interaction S51	**S30** Regulations of signaling pathways in energy metabolism and stress S53	**S37** Serial EM section analysis sheds light on brain microcircuit structure and function S54	Young Scientist Session	
12:10 – 13:30	Luncheon Seminar 4 (AstraZeneca)	Luncheon Seminar 5 (KRIBB)	Luncheon Seminar 6 (BIORCHESTRA Co., Ltd.)	Luncheon Seminar 7 (IUPS)		New (2023-2027) Council Meeting (12:20 – 15:00)			
13:30 – 14:20	**SL 7** **David Paterson** Disease in a dish using hiPSC: Transcriptional and signal transduction underlying neuromodulation of heart rhythm S8	**SL 8** **Armin Kurtz** Endo- and paracrine functions of the kidney interstitium S8							
14:20 – 16:20	**S31** Cardiac calcium signaling and electrophysiology S56	**S32** Toward finding therapeutic interventions: New avenues for understanding vascular pathophysiology S57	**S33** Glia physiology in brain function and diseases S59	**S34** Vascular inflammation S60	**S35** Physiological implication of membrane lipid dynamics S61		**S42** Novel treatment development for rare genetic disease S63	**S41** Stem cell-based disease modeling and cell therapy S64	
16:20 – 18:00									**Poster Presentation** Odd No. 16:20 – 17:10 Even No. 17:10 – 18:00
18:00 – 18:30	Break								
18:30 – 20:00	**Gala Party** Venue: Grand Ballroom (B)								

Day 4 November 4, Sat. Program at a Glance

KST (Korea Standard Time)	Room 325	Room 324	Room 323	Room 322	Room 321	Room 320	Grand Ballroom (A), 3F Lobby
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09:00 – 11:00	**S43** Molecular mechanism of membrane transport S66	**S44** Cutting-edge microscopy imaging technology in physiology research (Imaging) S67	**S45** Motility and smooth muscle conactility S68	**S46** Pathophysiology of trigeminal somatosensory system S70	**S38** Molecular mechanisms of ion channels in health and disease S71	**S48** Basic to translational science for neurodegenerative disease S72	Poster Viewing & Exhibition
11:00 – 11:10	**Break**						
11:10 – 12:00	**SL 9** **Walter Boron** Role of membrane proteins as channels for dissolved gases S9	**SL 10** **Tian Xue** Light and life – not just for seeing S9					
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13:30 – 14:30	**PL 3** **Lucio Cocco** Nuclear signalling: role of inositol lipids and inositide specific phospholipase C S6						
14:30 – 16:30	**S49** Exploring the cardioprotective mechanisms of regional diets to the human physiology S74	**S50** The Korean society of pharmacology S76	**S51** Adaptation to exercise training in health and disease S77	**S29** Glia control of brain function in health and disease S79	**S53** Stem cell therapy, technology and new potentials S80	**S54** New platform for cardiac safety evaluation of investigational drugs (CiPA session) S81	
16:30 – 17:00	**Closing Ceremony** Venue: Room 325						

CL1

The future of physiology is today

Denis Noble

University of Oxford, United Kingdom

The Central Dogma of Molecular Biology, and the Neo-Darwinist Modern Synthesis interpretation of Evolutionary Biology, sidelined physiology during the second half of the 20th century. Both are based on the four illusions of the Modern Synthesis (Noble 2021) and are invalid. It is now the mission of physiology to come to the rescue of both molecular and evolutionary biology. The roles of genes, and other molecular components, in function cannot be understood without physiological understanding of living systems and how they control the genome, while physiology is now understood to be the driver of evolutionary speciation (Noble & Noble, 2023).

Noble D. (2021). The Illusions of the Modern Synthesis. Biosemiotics. https://doi.org/10.1007/s12304-021-09405-3
Noble R. & Noble D. (2023). *Understanding Living Systems*. Cambridge: CUP

Keywords: physiology of evolution, Illusions of Modern Synthesis, future of physiology

CL2

Cardiac EC-coupling garden with Asian friends: the fruits we picked, much remains!

Martin Morad

Cardiac Signaling Center of USC, MUSC, and Clemson University, Charleston SC, USA

Cardiac EC-coupling field leap-forged forward with development of voltage clamp technic for cardiac muscle in early 60s and 70s as studies established that action potential while both triggering and controlling the duration of mammalian cardiac contraction, has evolutionarily not developed the trigger mechanism in frog or shark hearts but only the mechanism for direct control of contraction by action potential. The development of rapid cell-isolation technique for cardiomyocytes and using patch-clamp techniques identified Ca^{2+} current (I_{Ca}) to be the trigger for release of Ca^{2+} from the SR to activate contraction in mammalian hearts via a Ca^{2+}-induced Ca^{2+} release (CICR) mechanism. Ca^{2+} influx on Na^{+}/Ca^{2+} exchanger (NCX) and Ca^{2+} channel provided directly the transmembrane influx of Ca^{2+} for contraction in the frog or shark hearts that had little or vestigial SR Ca^{2+} stores. From 80s to the present, co-works with outstanding colleagues from China, India, Iran, Japan, South Korea, Taiwan and Europe enabled us to discover new physiological mechanisms: 1) Ca^{2+} channel signaling of the ryanodine receptors (RyR2) occurred through cross signaling of Ca^{2+} fluxes of the two channels but not by NCX, suggesting privileged access of DHPR to RYR2, 2) photo-release of Ca^{2+} triggers the release of Ca^{2+} but fails to terminate I_{Ca} triggered release, suggesting that higher released Ca^{2+} did not inactivate the release mechanism, 3) I_{Ca} triggered Ca^{2+} sparks originate from dyadic stores that reactivate in the same cellular location from beat to beat and develop into Ca^{2+} stripes along the t-tubular system, 4) in atrial cells, devoid of significant t-tubules, Ca^{2+} sparks were larger, lasted longer and occurred along the Z-lines faster than predicted by Ca^{2+} diffusion, 5) shear-stress can trigger Ca^{2+} sparks that may arise from mitochondria, 6) relaxant effects of beta-adrenergic agonist on the frog heart, devoid of significant SR Ca^{2+}-ATPase, was mediated by NCX, in sharp contrast to mammalian heart where the relaxation is mediated by Phospholamban/SERC2a regulation, and 7) NCX was bimodally regulated in shark hearts by adrenergic agonists. On-going multidisciplinary researches using CRSPR-Cas9 introduced mutations in Ca^{2+} binding site of human RyR2 that fully suppressed CICR revealed remodeling of EC-coupling such that other Ca^{2+} signaling pathways got activated that allowed cellular survival and beating. I am grateful to efforts of my Asian, American and European Scientists friend that have helped my search for the secrets of EC-coupling garden. The search continues for many of them as I celebrate their achievements. There is much to unravel.

Keywords: cardiac EC-coupling

PL1

Physiology and its transcriptional underpinning

Ronald Evans

Salk Institute, USA

The goal for cloning nuclear receptors for steroid and thyroid hormones, was to provide a means to study hormonal control of gene expression. Unexpectedly, we uncovered a Superfamily of 48 evolutionarily conserved receptors known as the Nuclear Receptor Superfamily. 42 of the related receptors represented harbored unknown functions which were termed orphan receptors. The characterization of these orphan receptors, the epicenter of the "Big Bang" era of molecular endocrinology. The resulting discoveries revealed an integrated and coordinated genome wide signaling networks as the essential underpinning of body physiology. In particular, the transcriptional regulation of these unrecognized physiologic pathways, led to breakthroughs that permitted novel ligand discoveries, the molecular basis of ligand-dependent transcription, and an on going expantion and what the future of this field may reveal.

Keywords: nuclear receptors, metabolism, pancreatic cancer, exercise mimetics, obesity

PL2

Neural mechanism of affective empathy

Hee-Sup Shin

Institute for Basic Science, Korea

Empathy, the ability to understand and share emotions of others, is crucial for social animals. It forms the foundation of various social behaviors, including emotional contagion, prosocial behavior, theory of mind, and perspective-taking. Observational fear, a form of emotional contagion is conserved among diverse animals including humans. Studying affective empathy in mice using the observational fear assay offers insights into the neural mechanisms underlying empathy. This talk will provide an overview of the status of current research on the neurobiology of empathy, focusing on the neural circuitry.

Keywords: empathy, emotional contagion, observational fear, social behavior, neural circuits

PL3

Nuclear signalling: Role of inositol lipids and inositid specific phospholipase C

Lucio Cocco, Matilde Y. Follo, Lucia Manzoli, Stefano Ratti

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Since 1987 (Cocco & Irvine, B.J., 248, 765-7015) evidence from several laboratories has highlighted the presence of autonomous nuclear inositol lipid metabolism. The evidence suggests that lipid signalling molecules are important components of signalling pathways operating within the nucleus. The findings are important given the fact that nuclear signalling activity controls cell growth and differentiation. Among the nuclear enzymes involved in this system, inositide-specific phospholipase C (PI-PLC) β1 has been one of the most extensively studied enzymes (Martelli & Cocco, Nature, 358, 242-45). Besides the studies on its signalling activity in physiological conditions, clinically oriented ones have shown that PI-PLCβ1 gene is associated with several pathological conditions. Nuclear PI-PLCβ1 is involved in the early stages of hemopoiesis and, namely, in the control of cell-cycle progression in progenitor hemopoietic cells. In addition nuclear PI-PLCβ1 plays a crucial role in the initiation of the genetic program responsible for muscle differentiation in that the enzyme activates the cyclin D3 promoter during the differentiation of myoblasts to myotubes. Down regulation of this enzyme is associated with progression of myelodysplastic syndromes (MDS) into acute myeloid leukaemia as well as with myotonic dystrophy or DM, both type 1 and type 2. Here we briefly highlight the most important evidence of the role of nuclear PI-PLCβ1 in these pathologies as well as the significance of subcellular localization of PI-PLCs. In addition it is quite clear the potential role of PLCβ1 as biomarker in high-grade gliomas.

Keywords: inositide-specific phospholipase C, inositol lipids, nucleus, signalling, disease

SL1

From passive "glue" to dynamic renovators: Active surveillance and remodelling of neuronal circuits by microglia and astrocytes

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The role of glial cells has become a major theme in studies investigating changes in neuronal circuits during development, learning, and recovery after damage. There is increasing appreciation for the significant contribution of glial cells to the remodeling of neuronal circuits, and thus their influence on various brain functions. In this talk, we will introduce two topics regarding the interactions between glia and neuronal synapses in the *in vivo* brain.
In the first topic, we will describe our current understanding of the functional significance of the dynamic motility exhibited by microglia. In the healthy mature brain, microglial processes regularly make contact with neuronal synapses. Such contact enhances excitatory synaptic transmission and promotes synchronous activity in local cortical circuits. In the damaged brain, the duration of microglia-neuron contact becomes prolonged, shifting from "touching" to "wrapping". The induction of epileptic action potentials and the resulting axonal swelling and sustained pathological depolarization triggers microglial processes to migrate to and enwrap the swollen axons. Following this, the neuronal membrane potential recovers to its normal resting potential which suggests that intense surveillance of damaged neurons by microglia serves to mitigate excitotoxicity.
In the second topic, we will describe the functional significance of the interactions between astrocytes/microglia and neurons in the context of chronic neuropathic pain. Here, peripheral nerve injury triggers maladaptive plastic changes throughout the somatosensory system (development phase), resulting in altered nociceptive signal processing that manifests as lasting tactile allodynia (maintenance phase). Underlying this in part, are alterations to the central nervous system driven by the initial injury-associated afferent barrage. Indeed, during the development phase, tactile stimulation of the injury site induces heightened responses from pyramidal neurons in the corresponding primary somatosensory cortex. Formation and elimination of dendritic spines here also becomes increased during this phase. Thus, heightened plasticity during the development phase facilitates the formation of putative pathological neuronal circuits. During the development phase, astrocytes show increased mGluR1/5 expression leading to heightened activity and release of thrombospondin-1, an important regulator of synaptogenesis. Notably, these phenomena can be leveraged to treat chronic neuropathic pain. Temporary pharmacological blockade of afferent input combined with re-activation of astrocytes permanently alleviates tactile allodynia. Mechanistically, this is correlated with the elimination of allodynia-associated dendritic spines. Interestingly, this astrocyte re-activation is accompanied by the activation of neighbouring microglia. This is mechanistically important as microglial ablation disrupts astrocyte-mediated treatment of allodynia.

Keywords: microglia, astrocyte, synapse, cortex, chronic pain

SL2

Molecular mechanisms of sudden cardiac arrest in young athletes: The case of ARVC

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ARVC (arrhythmogenic right ventricular cardiomyopathy) is an inheritable disorder characterized by a fibrofatty infiltration, predominantly ot he right ventricular free wall, arrhythmias and a high risk of sudden cardiac arrest, primarily during the concealed stage of the disease. Most cases of gene-positive ARVC result from mutations in genes coding for proteins of hte desmosome, and predominantly, for the gene coding for Plakophilin-2 (PKP2). Recent studies have demonstrated that exercise is a negative contributor to the progression of PKP2-dependent ARVC, both in the cardiomyopathic and the arrhythmic components. Studies from our laboratory have shown that the inceased risk of malignant arrhythmias with exercise may relate to a hyperphosphorylation of phospholamban, a key regulator of intracellular calcium homeostasis. Overall, our studies have shown that calcium dysregulation and triggered activity are primary mechanisms of arrhythmias in ARVC, and a key target for therapy.

Keywords: calcium, arrhythmias, sudden death, arrhythmogenic cardiomyopathy

SL3

Exploring novel lymphatics for brain clearance

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Understanding outflow of cerebrospinal fluid (CSF) is paramount for comprehending central nervous system (CNS) homeostasis and the pathogenesis of age-related neurodegenerative diseases, including Alzheimer's disease. The re-discovery of meningeal lymphatic vessels (mLVs) as a new route of CSF drainage has led to an explosive advance in our understanding of the regulation and roles of CSF outflow. Dysregulation of CSF outflow through mLVs exacerbates the phenotypes of Alzheimer's disease or delays recovery after stroke and traumatic brain injury in experimental animal models. Thus, the primary roles of mLVs are the drainage of CSF that contains neurotoxic protein aggregates, such as amyloid-β and tau protein, and brain antigens derived from pathologic and aged lesions and the drainage to the draining deep cervical lymph nodes. CSF in the subarachnoid space around the brain has long been known to drain through lymphatics to cervical lymph nodes, but the connections and regulation have been challenging to identify. In this meeting, I will introduce novel and main lymphatics for CSF outflow.

Keywords: cerebrospinal fluid, meningeal lymphatic vessels, brain clearance, aging, Alzheimer's disease

SL4

Pathophysiological functions of dual specificity phosphatase-2 in human diseases

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Constitutive activation of extracellular signal-regulated kinase (ERK) signaling pathway has been known to play a critical role in the pathogenesis of many diseases. In the past decades, most studies focused on identifying mutations of upstream stimulators of ERK cascade or abnormal activation of receptor tyrosine kinases. However, mutation cannot explain why abnormal activation of ERK cascade occurs in many kinds of cancers, even those without activating mutations. Our recent studies discovered that the expression of dual specificity phosphatase-2 (DUSP2), a nuclear phosphatase that inactivates ERK, was downregulated in human diseases such as cancer and endometriosis. Reduced expression of DUSP2 leads to prolonged activation of ERK and thus increases the transcription of numerous genes involved in angiogenesis, steroidogenesis, cell proliferation, immune suppression, cancer stemness, metastasis, immune cell infiltration, and drug resistance. Further studies reveal that DUSP2 is downregulated by hypoxia. As hypoxia is a common feature of various diseases, we infer that loss-of-DUSP2 is the key hub that mediates hypoxia-induced pathophysiological processes. Indeed, forced expression of DUSP2 or treatment with novel histone deacetylase inhibitors to restore DUSP2's function ameliorates hypoxia-induced disease malignancy. Taken together, these data demonstrate that cells evolve multiple processes to overcome the detrimental effect of low oxygen stress and DUSP2 serves as the key mediator to control these processes. Prevention of DUSP2 downregulation or restoration of DUSP2 expression may safeguard normal cellular functions or revert the disease pathophysiology.

Keywords: hypoxia, DUSP2, cancer, endometriosis, extracellular vesicles

SL5

Cellular specific abundance of dysferlin in skeletal muscle

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Dysferlin is an important protein, playing a role in maintaining skeletal muscle function. Mutations in the dysferlin gene result in one of two diverse dysferlinopathies, miyoshi myopathy and limb-girdle muscular dystrophy 2B. Both of these diseases present with muscle weakness and atrophy and are distinguished by the site of muscle weakness being distal limb-girdle and proximal lower limb girdle musculature, respectively. For most patients with dysferlinopathies, loss of lower leg muscle mass is evident in the gastrocnemius and soleus and muscle loss to all lower leg muscles is evident with the disease. Dysferlinopathies have recently been identified to include a metabolic phenotype at the cellular level.

Skeletal muscle is heterogenous in nature, being comprised of slow, oxidative through to fast, glycolytic fibres, typically distinguished by the presence of specific isoforms of myosin heavy chain (MHC), MHCI (type I), MHCIIa (type IIa) and MHCIIb/x (type IIb/x). They are different in their contractile speed and metabolic capacities. The work presented will explore the fibre specific abundance of dysferlin in mammalian skeletal muscle, and compare the relationship in associated fibre type specific differences in proteins important for mitochondrial abundance and glycogen regulation using calibrated western blotting.

Keywords: skeletal muscle, muscle disease, dysferlin, single fibres, muscle heterogeneity

SL6

Tentonin 3, a novel mechanosensitive channel with slow inactivatin kinetics: Its biophysical property and physiological functions

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Mechanotransduction is a biological process of the conversion of mechanical stimuli into biological responses. Numerous physiological functions such as touch, hearing, blood pressure control, proprioception, and pain require mechanotransduction processes. Mechanotransduction starts with mechanosensitive (MS) channels in many mechanosensory cells. In dorsal-root ganglion neurons, three distinct types of MS currents: rapidly-, slowly- (SA), and intermediately-adapting currents. Piezo channels confer rapidly-adapting currents. But, MS channels responsible for the SA-type MS currents were not known. Previously, we identified that Tentonin 3 (TTN3) elicits the SA-type MS currents, distinct from Piezo channels. As mechanically-activated channel, TTN3 mediates baroreceptor reflex, proprioception, and insulin-release from pancreatic beta cells.

Protein stoichiometric analysis revealed that TTN3 is a tetramer. TTN3 is a pore-forming channel subunit as it shows spontaneous and stretch-evoked currents when its purified proteins are incorporated to the lipid bilayer. TTN3 structure was predicted using deep-learning protein structure prediction programs coupled with molecular dynamics algorithms. The predicted TTN3 structure reveals a rectangular shape with a pore in the center. Four transmembrane a-helices (S1,2,5, and 6) face the lipid membrane, whereas, S3 and S4, comprise the wall of ion conducting pore. Mutations residues aligning the pore wall blocked MS channel currents. More importantly, a mutant of a conotoxin, NMB-1, once known as a blocker of the SA-type MS currents in dorsal-root ganglion neurons, inhibited TTN3, but not Piezo1. These results clearly indicate that TTN3 is a bona fide MS channel, not a regulator of Piezo1.

Keywords: Tentonin 3, TMEM150C, mechanosensitive, ion channel, structure

Special Education Lecture

Educating humanities in physiology education

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As everyone aware, Physiology is an essential area of study for all medical professionals. It covers a wide variety of systems at molecular, cellular, organ and entire individual levels. Traditionally, Physiology has been educated systematically according to the physiology textbook. However, such way of learning style may not be sufficient to solve real-world problems. Therefore, integrated teaching style under outcome-based education has been introduced in many universities all over the world. Through physiology education with integrated teaching style, learners can obtain knowledge that can be applied for real-world questions and some skills to conduct research. However, obtaining such knowledge and skills may not be sufficient for medical professionals. They also need to learn humanities, a social science to understand human's psychological, social, and cultural needs. It is not just for the duty for clinical/psychology class teachers, but for all course teachers including physiology teachers to teach humanities in each course. There are several ways to teach humanities in Physiology class. For example, clinical case study can be introduced under active learning/flipped class situation. In addition to discuss the physiological/pathophysiological mechanisms causing each symptom, learners can discuss what concerns each patient has, and how they communicate with patients to relieve pain. Followed by such discussion, role play can be also good practice for both knowledge integration and communication/attitude skill training. In physiology practice class, on the other hand, some class can be done in the skills lab in the hospital. Teachers can ask learners to wear properly and try to behave as if they are facing to real patients. In addition to introducing such integrated curriculum, students' behavioral assessment is also important. Learners and teachers should be aware that good academic performance is not enough to be a good professional. Students showing "unprofessional" behavior should be appropriately instructed. In summary, physiology education today is not just to learn how to think logically by integrating knowledges, but also to learn humanities as a part of medical education.

Keywords: teaching, case study, active learning

SL7

Disease in a dish using human-induced pluripotent stem cells: Transcriptional and signal transduction underlying neuromodulation of heart rhythm

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Background: Human induced pluripotent stem cells (hiPSCs) offer an unprecedented opportunity to generate a potentially unlimited source of cells to develop model systems of human disease, especially diseases with a genetic underpinning. Inherited arrhythmia syndrome is caused by cardiac channelopathies, with genetic mutations that alter the function of cardiac ion channels, which can lead to electrical disturbance and sudden cardiac death. Catecholaminergic polymorphic ventricular tachycardia (CPVT) causes a significant portion of lethal arrhythmia triggered by adrenergic stimulation. However, the molecular targets underlying the neuronal-cardiac communication remain unclear, and no therapeutic pathways have been validated against human tissue.
Method: Here we optimized current methods for induction of cardiac myocytes (hiPSC-CMs) and sympathetic neurons (hiPSC-SNs). We tested the hypothesis that a diseased phenotype also resides in CPVT neurons as well as cardiac myocytes. Mono-cultures and co-cultures were made from a patient with CPVT genotype (with isogenic pairing) to generate a model of triggered arrhythmia. hiPSC-CMs and hiPSC-SNs were characterized by immunofluorescence, flow cytometry and calcium (Ca^{2+}) imaging (Fura-2) and multi-array electrical mapping. Intracellular Ca^{2+} transients were measured in myocytes in response to isoprenaline, and in neurons in response to nicotinic stimulation. Single cell RNA sequencing of hiPSC-CMs, hiPSC-SNs and neurocardiac co-culture samples screened for potential target genes for CPVT.
Results: Healthy hiPSC-SNs possessed neurite outgrowth, stained positive for PHOX2B, tyrosine hydroxylase and peripherin. Derived myocytes showed spontaneous beating, stained positive for cardiac troponin T and a-actinin. CMs from the CPVT hiPSC line expressed a higher Ca^{2+} responsive to isoprenaline, caffeine and KCl stimulation when compared with healthy hiPSC-CMs. They also displayed spontaneous Ca^{2+} oscillations after isoprenaline. CPVT hiPSC-SNs had greater Ca^{2+} transients to nicotinic stimulation, indicating a diseased phenotype also resides in the neuron as well as the myocyte. Several gene transcripts in sympathetic neurons were markedly altered.
Conclusion: Using human iPSC derived cardiac myocytes and sympathetic neurons we have recapitulated many features of the anatomy and (patho)physiology as well as the transcriptomic atlas of these cell types from a patient with CPVT. Our data suggest that neuronal targeting to reduce sympathetic excitability may provide a therapeutic target in this vulnerable patient group.

Keywords: heart, sudden death, arrhythmia, stem cells, CPVT

SL8

Endo- and paracrine functions of the kidney interstitium

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Interstitial stellate cells in the kidney are endo- and paracrine regulators
The existence and functional relevance of stellate cells in nonneural

tissues has first been characterized in the liver. Stellate cells are resident interstitial cells which express PDGFR-β. In organ threatening situations stellate cells transform into collagen producing myofibroblasts what is considered as an organ protective mechanism. Meanwhile it has become clear that stellate cells are not only organ stabilizers and protectors but also signal generators that regulate organ and body function under physiological conditions also in other organs. This function has become very evident for the kidney, where stellate cells form the majority of resident interstitial cells. Those PDGFR-ß expressing cells are found in all kidney zones. There, stellate cells can be grouped in cell populations capable to express erythropoietin which is the bodies main regulator of erythropoiesis. Another subpopulation (outside the juxtaglomerular apparatus) expresses prorenin which can be converted to renin or can locally activate prorenin receptors on tubules. Another subpopulation expresses cyclooxygenase 2 producing prostaglandins which regulate intrarenal tissue perfusion and tubular function. A further subpopulation of stellate cells expresses proenkephalin (PENK) which intrarenally acts on opioid receptors. Common to these endo/paracrine stellate cells in the kidney is, that they do not store their signal products but apparently produce them upon demand. Another common characteristic of the cells is, that they regulate the production of the respective signals by (de)recruitment of producing cells instead by up- and downregulation in fixed cell populations. The lecture aims to introduce and to characterize renal stellate cells as endo-or paracrine cells and to demonstrate how they respond to a damage threat to the kidneys.

Keywords: stellate cells kidney, erythropoietin, prorenin, prostaglandins, PENK

SL9

Role of membrane proteins as channels for dissolved gases

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The dogma had been that all gases cross biological membranes simply by dissolving in and then diffusing through the lipid phase of membranes (solubility-diffusion theory). However, the last two decades have seen a major paradigm shift, with the discoveries of (1) gas-impermeable biological membranes and (2) membrane proteins ("gas channels") that conduct CO_2, NH_3, NO, and/or O_2. Cell- and whole-animal physiological studies on a wide range of such channels, together with mathematical simulations, X-ray crystallography, and molecular-dynamics studies have begun to clarify the molecular mechanisms of two gas-channel families: the tetrameric aquaporins (AQPs) and trimeric Rhesus (Rh) proteins. Gas channels can have biological impact only if the "background" membrane permeability is relatively low. Some investigators have studied CO_2 diffusion through planar-lipid-bilayer systems comprising (a) highly fluid lipids, (b) considerable decane content within the bilayer, and (c) an annulus of nearly pure decane surrounding the bilayer. Not surprisingly, such systems have extraordinarily high CO_2 permeabilities—with diffusion constants ($D_{M,CO2}$) approaching 10% of what one would expect for a membrane made up of water. Here, addition of channels cannot augment CO_2 fluxes. On the other hand, measurements on red blood cells (RBCs) and *Xenopus* oocytes indicate that—in the absence of functional gas-channels—membrane permeabilities to CO_2 or O_2 are extremely low, with $D_{M,CO2}$ low as 0.002% of membrane made up of water.

In this lecture, I present a series of biophysical, physiological, and pathophysiological vignettes describing recent work on : (1) the molecular mechanisms of CO_2 movement through AQPs, (2) two potentially new gas-channel families, (3) the role of AQP1 in HCO_3– reabsorption by renal proximal tubules, (4) roles of AQP1 and the Rh complex as O_2 channels in RBCs, (5) potential roles of these RBC channels in exercise, and (6) the potential roles of AQP5 and AQP1 in pulmonary O_2 toxicity (POT).

We will see that different AQPs exhibit characteristic selectivities for various gases over water, that point mutations can confer or eliminate AQP gas permeability, and that blocking agents can selectively block CO_2 diffusion through either the four monomeric pores or the central pore between the monomers. In mammalian RBCs, nearly all CO_2 and O_2 move through membrane channels, and deleting these channels appears to have a surprising impact on exercise tolerance. Finally, the genetic deletion of AQP5 and AQP1 may ameliorate POT in a mouse model.

Keywords: carbon dioxide (CO_2), oxygen (O_2), aquaporins, rhesus (Rh) proteins

SL10

Light and life – not just for seeing

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Light sensation not only provides us with the image vision perceptions, but also regulates many physiological functions, such as circadian rhythm, pupillary reflex, arousal, mood, development and metabolic homeostasis. But the photoreceptors, neural circuits, molecular and cellular mechanisms of these light regulated life processes are still largely unknown.

Recently, our laboratory discovered the neurophysiological mechanisms of light-at-night induced depression; cortical synaptogenesis promoted by light sensation during infanthood; and even light regulated glucose metabolism. These works revealed that the interaction between "light and life" is much more extensive and important than we generally understood.

Keywords: ipRGC, non-image forming, melanopsin, neural circuits

S1-1

Molecular mechanism of selective ion permeation through the K^+ channels: A computational study

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Ion channels are molecular entities that produce many physiological functions including nerve conduction and muscle contraction. K^+channels, in particular, are critical for maintaining the resting potential and generating the action potential. For these roles, rapid and selective ion permeation is significant. Historically, ion permeation rates and selectivity have been measured by the electrophysiological experiments such as the patch clamp technique. The measured current amplitudes are typically tens of pA, meaning that one K^+ ion permeates in 10 ns in average. Most textbooks state that the K^+ selectivity of the K^+ channels is 10,000-fold over Na^+. To fully understand the mechanism of these rapid and selective permeation, it is necessary to observe molecular motions. Experimental observation is still impossible, but using computers, virtual observation is now possible. Molecular dynamics (MD) simulation is a technique to animate molecular motions at the atomic level by numerically solving the Newton's equation of motion. MacKinnon determined the x-ray crystallographic structure of the K^+ channel, which opened the way for computational visualization. In my talk, I will briefly review the previous results by MD simulations aimed at revealing the molecular mechanism of ion permeation and selectivity through the K^+channels. All previous papers claimed that the knock-on (or direct knock-on) mechanism is important for the rapid ion permeation and that the snug-fit model is a key to high selectivity. In the knock-on mechanism, an ion located in the channel is electrically repelled by the next incoming ion; in other words, the next ion is necessary for ion permeation. This is to cut the high affinity between K^+ ions and the K^+channels due to the snug-fit model, in which carbonyl oxygen atoms tightly fit to the K^+ ions in the channel. However, our conclusion based on the detailed analyses of ion trajectories are opposite to these previous findings. We found that the knock-on mechanism not only cannot explain the rapid permeation mechanism, but is not necessary for permeation. One ion in the channel flows out of the channel before the next ion arrives. This means the affinity of K^+ ions to the K^+ channel is not high, casting doubt on the snug-fit model or high selectivity. Indeed, the selectivity defined by the conductance ratio of K^+ to Na^+ obtained by the MD simulation was 38.5, which was confirmed by the single-channel recordings, where the ratio was 78.3. Note that even at this low selectivity, the reversal potential is −94mV (38.5) or −112 mV (78.3), which is sufficient to maintain the resting potential. Furthermore, the computed free energy barrier for Na^+entry was found to be high due to the dehydration penalty, which is important to reject most Na^+. In summary, the affinity of K^+ to the K^+channel or selectivity is low, which contributes to the rapid permeation. Thus, our findings call for updating our knowledge of the selective ion permeation mechanism at the atomic scale.

Keywords: ion channel, molecular dynamics, knock-on, snug-fit, selectivity

S1-2

Molecular dynamics simulations of calcium ion channels with a multisite calcium model

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We developed a multisite calcium ion model in the framework of the non-polarizable CHARMM force field (Nat Commun 2020, 11:922), which can quantitatively reproduce the interactions between calcium ions and proteins in all-atom molecular dynamics (MD) simulations. With this model, we investigated the ion permeation and selectivity of multiple calcium-permeable ion channels, including ryanodine receptors, TRPVs, and Ca_v, which exhibit diverse ion permeability and selectivity properties. Our simulation results suggest that the Ca^{2+} selectivity is mostly achieved by electrostatic effects. The shape and the charge distribution of ion channels result in specific Ca^{2+} binding sites and distinct binding strengths, which appear to be the key factors that determine the Ca^{2+} selectivity. The multisite calcium model has been implemented in the CHARMM-GUI server to facilitate Ca^{2+}-related MD simulation studies.

Keywords: calcium channel, molecular dynamics, ion model, permeation, selectivity

S1-3

Molecular dynamics simulation and structural analysis of the divalent cation blocking mechanism generated in prokaryotic cation channel

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Neural activity controls critical biological responses, such as thinking, memory formation, and muscle contraction. The transmission of neural activity occurs through various cation-selective channels on the nerve cell membrane. Divalent cation blocking is observed in important tetrameric cation channels. For example, magnesium ions block the current of the NMDA receptor in a voltage-dependent manner. The pore domain of the tetrameric cation channels consists of two transmembrane helices, and the selectivity filter (SF) locates in the loop between them. The loop also contains one or two pore helices (P1 and P2 helix), and the SF follows the P1 helix. As the name indicates, the SF of the tetrameric cation channels is responsible for the selective permeation of cations. For NMDA receptors, functional analyses and simulations suggest that the residues at the SF are involved in magnesium inhibition. However, the detailed molecular mechanism of divalent cation blocking on the selectivity filter is not fully understood. The divalent cation blocking is not observed in tetrameric channels, including typical voltage-gated sodium channels (Navs), whose structures have been well studied. Therefore, reproducing the blocking on Nav and its structural analysis would help elucidate their molecular mechanisms. Prokaryotic Navs (BacNavs) were cloned from bacteria living in various environments and characterized, providing many insights into sodium channels' molecular basis. As well as other sodium channels, BacNavs do not show divalent cation blocking. NavAb is the most critical contributor to the first full-length structure of Nav at atomic resolution. By introducing some single mutations into the NavAb SF, we successfully reproduced the divalent cation-blocking effect on NavAb. Using these mutated NavAb, we conducted structural analysis and molecular dynamics simulation to elucidate mechanisms of the divalent

cation blocking. The small-side chain mutation of the selectivity filter residue creates a small cavity for an extra water molecule. It enables the coordination of divalent cations via a water molecule in the ion pathway. Therefore, the sodium ions were repelled by the giant positive field on the bottom of the selectivity filter.
Knowing the molecular basis of the divalent cation block of widespread biologically important channels is possible. Furthermore, the generation of a new function by single-point mutation is reminiscent of the evolutionary process of channels.

Keywords: ion channel, prokaryotic sodium channel, crystal structure, electrophysiology, molecular dynamics simulation

S1-4

Computer simulations of voltage gated sodium channels: from ion conduction to drug discovery

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This study will describe how molecular simulations have helped address three aspects of voltage gated sodium channel (Nav) function: ion permeation and selectivity, modulation by phosphoinositides, and binding of channel blocking drugs.
Nav create the upstroke of action potentials in excitable cells by opening a Na^+ selective transmembrane pore in response to small depolarising signals. These channels are able to rapidly move Na^+ ions down their electrochemical gradient, while limiting the passage of other ions. But how this selection for ions occurs remains difficult to explain. Recent experiments show that Nav channel kinetics and voltage dependence are modulated by phosphoinositides (PI(4,5)P2). While this is likely to occur via a direct interaction with Nav, the structural basis of phosphoinositide modulation remains to be understood. Small molecule inhibitors of Nav are common pharmacological agents used to treat a variety of cardiac and nervous system pathologies. They bind within the pore to directly block the conduction pathway and/or stabilize a non-conductive state, and many have been suggested to access this site via lateral lipid filled fenestrations. Despite their abundant clinical use, we lack specific knowledge of how they access the pore and the details of the drug binding sites.
Here we demonstrate a mechanism by which bacterial sodium channels distinguish between ions, but show the limitations in using classical simulations to address this for eukaryotic channels. We identify a putative phosphoinositide binding site and a potential mechanism for channel regulation. Finally, we present a molecular analysis of how 10 drugs with different structures and clinical uses access and bind to the pore cavity of the sodium channel, testing the potential to target channel subtypes and conformational states by differential access to the drugs to the pore binding site.

Keywords: sodium channels, molecular dynamics, ion permeation, lipid regulation, drug-protein interactions

S2-1

A new paradigm for the role of lactate as an important metabolic signal regulating mitochondrial biogenesis

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Our understanding of lactate has changed drastically in recent years. Once considered to be a metabolic waste product and a cause of fatigue, lactate is now recognized as a useful carbohydrate energy source. Intriguingly, growing evidence shows that lactate acts as a signaling molecule that induces physiological adaptations. In this session, acute and chronic responses to manipulation of lactate concentration on mitochondrial content and respiratory function in skeletal muscle will be presented. The result of single lactate administration showed that mitochondria-related gene expressions in skeletal muscle increased with or without exercise. Moreover, daily lactate administration enhanced mitochondrial enzyme activities in skeletal muscle. These results highlight that lactate is a signaling molecule that induces mitochondrial adaptations, and that training effects would be enhanced by an increase in lactate concentration. Emerging evidence shows that mitochondria change not only content, but also respiratory function. Then, we explored mitochondrial respiratory function using isolated mitochondria. Daily lactate administration altered substrate-dependent mitochondria-specific oxygen consumption rate, along with increases in enzyme activities and protein contents of respiratory chain complexes in skeletal muscle. Given the potential role of lactate as a food additive, we also examined the effects of oral lactate administration on mitochondria adaptations in skeletal muscle. The results suggest that oral lactate supplementation would enhance mitochondrial enzyme activity. Finally, this presentation will highlight how these data help us understand how to interpret lactate concentration in athletic settings.

Keywords: lactate, mitochondria, skeletal muscle, exercise

S2-2

Using 'omics' to understand mitochondrial adaptations to different types of exercise

David Bishop

Victoria University, Australia

Given the importance of mitochondrial biogenesis for skeletal muscle performance and health, considerable attention has been given to understanding the molecular changes that help to determine mitochondrial adaptations to exercise. This talk will focus on published and unpublished 'omics data that provide new and exciting insights into the many molecular changes that contribute to exercise-induced mitochondrial adaptations. The results of RNA sequencing (RNA-seq) based transcriptomics highlight how different mitochondria-related gene transcripts respond following different types of exercise, and how this may help to explain divergent mitochondrial adaptations to different types of exercise training. These RNAseq results also indicate that there are transcriptional responses that are shared across different exercise prescriptions. Of particular interest is how exercise-induced mitochondrial damage activates transcriptional pathways associated with mitochondrial stress and how this may help to explain the powerful effects of very high-intensity exercise to improve mitochondrial respiratory function. The results of training studies incorporating whole-

muscle proteomics will then be used to highlight an intricate and previously unknown network of differentially prioritised mitochondrial adaptations that occur in response to different types of training. It will be shown that changes in hundreds of transcripts, proteins, and lipids are not stoichiometrically linked to the overall increase in mitochondrial content. Finally, the results of single-fibre proteomics show how exercise intensity influences fibre recruitment and ultimately induces fibre-specific changes in mitochondrial proteins that can help to explain how different types of exercise induce divergent mitochondrial adaptations. Finally, this presentation will highlight how these exciting new tools can help exercise and sport scientists to better understand how best to prescribe exercise to achieve specific mitochondrial adaptations. In summary, this session will provide an important update on how different physiological stresses help create new normal mitochondria. The target audience will be both exercise and sport scientists with an interest in the mechanisms that underlie adaptations to exercise training.

Keywords: mitochondria, exercise, RNA-seq, proteomics, skeletal muscle

S3-1

Extracellular vesicles and arrhythmia; a diagnostic biomarker and treatment

Boyoung Joung

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Extracellular vesicles, such as exosomes, are small membranous vesicles secreted all cell types. These vesicles enclose cytosolic proteins and nucleic acids, including mRNA, microRNA (miRNA), and noncoding RNAs, and play important roles in cell-to-cell communication and biologic functions. Exosomal long noncoding RNAs (lncRNAs) are known as ideal diagnostic biomarkers of various diseases.
Atrial fibrillation (AF), the most common type of cardiac arrhythmia, is thought to be regulated by changes in miRNA and lncRNA expression. Recently, we found that Forty-five miRNAs were expressed significantly higher (>1.5-fold) in patients with persistent AF, but not in patients with paroxysmal AF, relative to the levels in patients with SVT control. Notably, expression of 5 miRNAs (miRNA-103a, -107, -320d, -486, and let-7b) was elevated by more than 4.5-fold in patients with persistent AF. These findings suggest that serum exosomal miRNAs might be used as novel biomarkers to reflect the progression of AF. We also found that exosomal lncRNAs LOC105377989 and LOC107986997 were consistently upregulated in the serum of patients with persistent AF compared with controls (P <.0001). Moreover, both exosomal lncRNAs exhibited significant diagnostic validity for AF. Notably, exosomal lncRNA LOC107986997 was involved in AF-related pathophysiological mechanisms.
Small EVs (sEVs) might contribute to the pathogenesis of AF. We investigated the role of sEVs derived from patients with persistent AF in the pathophysiology of AF. AF-sEVs treatment reduced cell viability, caused abnormal $Ca2^{+}$ handling, induced reactive oxygen species (ROS) production and led to increased CaMKII activation of non-paced and paced atrial cardiomyocytes. qRT-PCR experiment identified that miR-30a-5p was significantly down-regulated in AF-sEVs, paced cardiomyocytes, and atrial tissues of patients with persistent AF. CaMKII was predicted by bioinformatics analysis as a miR-30a-5p target gene and validated by a dual luciferase reporter; hence, we evaluated the effects of miR-30a-5p on paced cardiomyocytes and validated miR-30a-5p as a pro-arrhythmic signature of AF-sEVs. Consequently, AF-sEVs-loaded with miR-30a-5p attenuated pacing-induced Ca^{2+}-handling abnormalities, whereas AF-sEVs-loaded with anti-miR-30a-5p reversed the change in paced cardiomyocytes. Taken together, the regulation of CaMKII by miR-30a-5p revealed that miR-30a-5p is a major mediator for AF-sEVs-mediated AF pathogenesis. Accordingly, these findings suggest that sEVs derived from patients with persistent AF exacerbate arrhythmogenesis via miR-30a-5p.

Keywords: atrial fibrillation, microRNA, extracellular vesicles, Biomarker, Diagnosis

S3-2

Pathophysiological roles of a cardiac potassium channel in ventricular repolarization

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A cardiac potassium channel, the I_{Ks} channels composed of the alpha subunit KCNQ1 and beta subunit KCNE1, contributes to the repolarization phase of the cardiac action potential. Mutations in these genes are associated with the development of lethal arrhythmias due to congenital QT prolongation syndrome and are influenced by sympathetic nerve stimulation and sex hormones. As a molecular mechanism, we have demonstrated the involvement of a molecular complex of the KCNQ1 channel in the IKs regulation by intracellular Ca^{2+}, cAMP [1], and NO [2]. Recently, our proteomic analysis for binding partner of KCNQ1 revealed that the KCNQ1 molecular complex involves in Ca^{2+} signaling, epithelial junction signaling, mitochondrial dysfunction and so on, but the pathophysiological role has not been elucidated. Therefore, we aimed to test whether I_{Ks} channels activated by pathological Ca^{2+} overload may compensate for arrhythmias using genetically engineered (I_{Ks}-Tg) mice (8-24 weeks, genetic background: C57BL6J) expressing cardiac human I_{Ks} channels (tandem protein of KCNE1 and KCNQ1). We employed a sepsis model (Cecal Slurry (CS) intraperitoneal injection method) for pathological Ca^{2+} overload condition. We found that the sepsis score I_{Ks}-Tg male mice (n = 5) was significantly lower than that in age-matched wild-type male mice (n = 15). To seek the underlying mechanisms, effects of CS on action potential traces recorded from patch-clamped ventricular myocytes were compared between I_{Ks}-Tg mice and wild type mice. In wild-type male mice, APD was prolonged 1-day after CS injection, indicating that CS altered regulation of cardiac repolarization. However, the CS injection did not alter APD in the I_{Ks}-Tg male mice. These results suggest a protective role of the I_{Ks} channel on cardiac pathological modification by sepsis.
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Keywords: potassium channel, sepsis, action potential

S3-3

Stretch-induced sarcoplasmic reticulum calcium leak promotes atrial and sinoatrial dysfunction

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Aims: Despite numerous reports documenting an important role of hypertension in the development of atrial fibrillation (AF), the detailed mechanism underlying the pathological process remains incompletely understood. Here, we aim to test the hypothesis that diastolic sarcoplasmic reticulum (SR) Ca^{2+} leak in atrial myocytes, induced by mechanical stretch due to elevated pressure in the left atrium (LA), plays an essential role in the AF development in pressure-overloaded hearts.
Methods and results: Isolated mouse atrial myocytes subjected to acute axial stretch displayed an immediate elevation of SR Ca^{2+} leak. Using a mouse model of transverse aortic constriction (TAC), the relation between stretch, SR Ca^{2+} leak and AF susceptibility was further tested. At 36 hours post TAC, SR Ca^{2+} leak in cardiomyocytes from the LA (with hemodynamic stress), but not right atrium (without hemodynamic stress), significantly increased, which was further elevated at 4 weeks post TAC. Accordingly, AF susceptibility to atrial burst pacing in the 4-week TAC mice were also significantly increased, which was unaffected by inhibition of atrial fibrosis or inflammation via deletion of galectin-3. Western blotting revealed that type 2 ryanodine receptor (RyR2) in LA myocytes of TAC mice was oxidized due to activation and upregulation of Nox2 and Nox4. Direct rescue of dysfunctional RyR2 with dantrolene or rycal S107 reduced diastolic SR Ca^{2+} leak in LA myocytes and prevented atrial burst pacing stimulated AF.
Conclusion: Our study demonstrated for the first time the increased SR Ca^{2+} leak mediated by enhanced oxidative stress in LA myocytes that is causatively associated with higher AF susceptibility in pressure-overloaded hearts.

Keywords: hemodynamic stress, stretch, calcium leak, oxidation, atrial fibrillation

S3-4

Effects of a novel RyR2 selective inhibitor on malignant arrhythmias in CPVT mouse models

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Genetic mutations in the ryanodine receptor type 2 (RyR2) can cause life-threatening arrhythmias and more than 300 of disease-associated mutations have been reported to date. Among the arrhythmic diseases caused by RyR2 gain-of-function mutations, catecholaminergic polymorphic ventricular tachycardia (CPVT) is the most common. Conventional antiarrhythmic drugs, i.e., Na channel inhibitors, Ca channel inhibitors, ß-blockers, used to treat CPVT sometimes fail to suppress arrhythmias, and implantable cardioverter defibrillators (ICDs) could exacerbate arrhythmias. Reducing RyR2 activity is thought to suppress arrhythmias in CPVT, but there are no clinically available antiarrhythmic drugs with RyR2-specific inhibitory action. We developed a high-affinity and selective RyR2 inhibitor based on hit compounds identified by a high-throughput screening. This compound successfully suppressed arrhythmias in CPVT mouse models harboring mutant RyR2s. Unlike conventional anti-arrhythmic drugs, this compound did not affect ECG parameters at the effective doses. Our results demonstrate that the specific suppression of RyR2 activity is highly effective in preventing and terminating arrhythmias caused by RyR2 hyperactivation.

Keywords: ryanodine receptor, arrhythmia, anti arrhythmic drugs, Ca^{2+} waves, cardiac

S3-5

Adrenergic mechanisms that partially suppress CPVT constitutively

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Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a condition typically begin in childhood and characterized by arrhythmia in response to physical activity or emotional stress. β-adrenergic receptor (βAR), a prototypical G-protein coupled receptor (GPCR) that play pivotal roles in sympathetic regulation, mediates the adrenergic signaling leading to CPVT. In heart cells, β1AR signaling mediates cAMP signaling globally, while β2AR signaling is confined within the vicinity of cell membrane and T-tubules. Recently we found that stimulation of β2AR compartmentalizes β1AR signaling into nanoscale local domains in a phosphodiesterase-4-dependent manner by GPCR kinase-2 (GRK2)-mediated phosphorylation of β1AR C-terminus. Stimulation of β2AR suppresses the arrhythmogenesis in CPVT models. A knock-in (KI) rat model harboring mutations of the GRK2 binding site of β1AR C-terminus becomes arrhythmogenic in response to catecholamine stimulation. Cardiomyocytes carrying both KI and CPVT mutations exhibits more frequent intracellular calcium waves and triggered activities than cells with a CPVT mutation only. The β2AR-induced offside compartmentalization of β1AR provides a constitutive "negative feed-forward" mechanism that serves to stabilize electrical activities of cardiomyocytes during adrenergic signaling.

Key Words : arrhythmia, adrenergic receptor, calcium signaling

S4-1

LncRNAs at the synapse: Implications in synaptic plasticity and memory

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Long non-coding RNAs is emerging as a key regulatory RNA in nervous system. However, physiological relevance of this regulatory RNA in sculpting neural circuits and governing input-specific cognitive functions via spatial resolution of gene expression control remains elusive. We have performed a genome-wide transcriptomics analysis using total RNA purified from synaptodendritic compartment from hippocampus to identify synapse-enriched lncRNAs. We have highlighted a hitherto unknown regulatory functions of lncRNAs that operates at spatial scale to regulate synaptic plasticity and memory. Furthermore, our study demonstrated that a specific subset of these lncRNAs are modified during early life stress leading to memory deficits.

Keywords: memory, synaptic plasticity, long non-coding RNA

S4-2

Forebrain-deletion of m6A reader YTHDF3 modulates mouse adaptive behaviors reared in enriched environment

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Enriched environment (EE) housing has enduring effects on animal behavior, which requires de novo transcription and translation in neurons. Although significant research has been done to understand the underlying transcriptional regulation through neuroepigenetics, the potential involvement of RNA epitranscriptomic regulation in shaping an animal's adaptive behavior to EE remains unexplored. This study investigates the impact of 7 weeks of EE while concurrently deleting YTHDF3 protein in the forebrain of adult male C57BL/6 mice. YTHDF3 is a reader protein that specifically binds to N6-methyl-adenosine (m^6A), the most abundant modification on messenger RNAs in the mammalian brain. Knocking down YTHDF3 in dissociated cultures of rodent hippocampal neurons induced abnormal spine phenotypes. In the current study, YTHDF3-depletion in mature excitatory neurons in the mouse forebrain leads to the manifestation of multiple phenotypic deviations from the behavior of wild-type mice, both in environment-dependent and independent manner. Unlike effector proteins that are functionally required for neuronal development and transmission, whose deficiency often results in animal's failures in performing behavioral tests, the absence of YTHDF3 results in behavioral modifications that are highly flexible and context-dependent. This finding underscores the modulatory role of the YTHDF3-dependent molecular pathway and establishes a novel molecular connection between the m6A regulatory pathway and the housing effects on the behaviors of adult mice.

Keywords: rotarod test, anxiety, stress coping, social interaction

S4-3

Deciphering the neural epitranscriptome: The roles of mRNA modification in neurodevelopment

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Proper development of the nervous system is critical for its function, and deficits in neural development have been implicated in many brain disorders. Recent discoveries of widespread mRNA chemical modifications raise the question of whether this mechanism plays a post-transcriptional regulatory role in the development and function of the brain. N6-methyladenosine (m^6A), installed by the Mettl3/Mettl14 methyltransferase complex, is the most prevalent internal mRNA modification that controls various aspects of mRNA metabolism, including stability, translation, splicing, and localization. Neurons are distinctly polarized cells where mRNA can be transported and localized in distal structures like axons and dendrites. However, how m^6A modification influences such RNA localization in developing neurons has not been understood well. We showed that *Mettl14* deletion in postmitotic neurons diminished m^6A content and impaired axonal projection during corticogenesis. RNA-seq analysis and single molecule in situ hybridization experiments revealed subsets of mRNA targets were mislocalized in the neurites of postmitotic neurons with m^6A loss-of-function. Further, we identified YTHDF2 as the reader protein responsible for mRNA transportation and localization in interhemispheric callosal axons in the developing brain. Our study will enlighten the epitranscriptomic mechanism to regulate axon projection and guidance during mammalian cortical neurogenesis.

Keywords: epitranscriptomics, RNA translocation, m6A, neurodevelopment, axon projection

S5-1

Neuronal regulation of adult taste stem cells

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Taste bud cells turn over continuously throughout life. Taste tissue homeostasis is maintained by the replacement of senescent taste cells with new ones generated from adult taste stem cells. This process requires innervation, which was first noted more than a century ago. Until recently, the molecular basis of neuronal regulation of adult taste stem cells was unclear. Prompted by our observation that single $Lgr5^+$ taste stem/progenitor cells give rise to all different types of mature taste cells in an *ex vivo* culture system ("taste organoids") in the absence of nerve input, we hypothesized that one of the components in the defined culture medium may be the principal gustatory neuron-produced factor. We set out to identify such factor. We focused on R-spondin, the ligand for the Lgr5 receptor. Using in situ hybridization, we show that R-spondin 2 (Rspo2) is expressed in gustatory neurons. Using a gustatory nerve transection model and adenovirus-encoded R-spondin, we show that exogenous R-spondin substitutes for neuronal input for taste cell generation. Using the organoid culture system, we further show that R-spondin is required for taste cell generation. Consistent with the *ex vivo* data, the number of taste buds is reduced in a hypomorphic mouse model of Rspo2 to half of that in wild-type control mice. R-spondin interacts not only with Lgr5 or its analogs Lgr4/6 but also with two E3 ubiquitin ligases Rnf43/Znrf3 to regulate Wnt signaling (Lgr4/5/6 – positive regulators, Znrf3/Rnf43 - negative regulators). Therefore, we hypothesized that Rnf43/Znrf3 may serve as a brake, controlled by gustatory neuron-produced R-spondin, for maintaining taste tissue homeostasis. Consistent with this model, taste cell hyperplasia and exuberant generation of ectopic taste buds occur in mice lacking Rnf43/Znrf3 in taste stem/progenitor cells, mirroring the effect of exogenous R-Spondin. We further demonstrate that ablating Rnf43/Znrf3 renders neuronal regulation of taste tissue homeostasis dispensable: regeneration of taste cells occurs in the double knockout mice even in the nerve transection model. In summary, our data suggests that the interaction of gustatory neuron-produced R-spondin and adult taste stem cell-expressed Rnf43/Znrf3 plays a central role in neuronal regulation of taste stem cells.

Keywords: R-spondin, taste stem cells, Rnf43, Znrf3, gustatory neurons

S5-2

Molecular and cellular mechanisms of sodium taste

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Understanding mechanisms underlying the taste of sodium (sodium taste) is crucial in combating health problems associated with excessive salt consumption in modern societies. Currently, the epithelial sodium channel (ENaC), composed of three essential subunits (α, β, γ), is considered to be the sodium taste sensor in taste cells. However, there is controversy regarding whether taste cells express all three ENaC subunits, which leaves the mechanisms of sodium sensing unresolved. In this study, we employed full-length single-cell RNA sequencing of fungiform and circumvallate taste buds and discovered taste cells that express all ENaC subunits, specifically in fungiform taste buds. We observed reporter protein expression driven by the intersectional control of the ENaCα and β promoters in a subset of fungiform taste cells, but not in circumvallate taste buds, providing further evidence of ENaC subunit co-expression. Additionally, in mice expressing channelrhodopsin-2 in these cells, blue light (445 nm) illumination of the tongue selectively activated sodium-selective neurons in the nucleus tractus solitarius and effectively mimicked the behavioral responses to sodium taste. These transcriptomic and functional findings not only offer a precise identification of sodium taste cells and their function but also provide insights into their fate specification program.

Keywords: taste, sodium, salt, transcriptome, optogenetics

S5-3

Wiring the taste system

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Animals use taste information to evaluate the nutritional value (sweet, umami, salty) and safety (bitter, sour) of food. Because this assessment is critical for the survival of the animal, taste cues immediately cause stereotypical behaviors (attraction vs aversion) and evoke innate affect (pleasure vs disgust). The taste data gathered by taste ganglia is then transmitted to the rostral nucleus tractus solitarius (rNTS) located in the medullary brainstem. This nucleus acts as a central hub where taste signals converge with other neural inputs from external senses (such as texture, odor, temperature) and internal sensations (like hunger and thirst). Subsequently, this integrated information is relayed to various brain regions. This intricate taste sensory system provides an excellent avenue for investigating how the brain accurately processes external stimuli and initiates innate behaviors. It is worth noting that even decerebrate animals or anencephalic babies, who lack most of the forebrain and midbrain structures, maintain specific reflex responses (tongue protrusion, retching) and salivation in response to taste. This suggests that the connections between taste and these reflex circuits are hardwired within the brainstem. In this presentation, I will delve into our recent progress in uncovering the brainstem circuits responsible for orchestrating taste-related behaviors.

Keywords: taste, brainstem, circuitry, salivation

S5-4

Identification of postprandial sodium sensor in the *drosophila* gut

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Sodium is essential for all living organisms. Animals including insects and mammals detect sodium primarily through peripheral taste cells. It is not known, however, whether animals can detect this essential micronutrient independently of the taste system. Here, we report that *Drosophila IR76b* mutants that were unable to detect sodium became capable of responding to sodium after a period of dietary salt deprivation. From a screen for cells required for the deprivation-induced sodium preference, we identified a population of anterior enteric neurons that we named INSO (Internal Sodium Sensing) neurons, that are necessary and sufficient for directing a behavioral preference for sodium. Enteric INSO neurons innervate the gut epithelia mainly through their dendritic processes and send their axonal projections along the esophagus to the brain and to the crop duct. Through calcium imaging and CaLexA experiments, we found that INSO neurons are amiloride-insensitive and respond immediately and specifically to sodium ions. Taken together, we have identified a previously unknown taste-independent sodium sensor that is essential for the maintenance of sodium homeostasis.

Keywords: enteric neurons, drosophila, postprandial sodium sensor, INSO neurons

S6-1

Prevention of occupational health disorders in Indian informal sectors: A participatory approach

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Informal or unorganized sectors are those, which are not under any governmental regulations, and thus the people involved in this profession are not covered under any benefits like leaves, retirement, sickness or the benefits of stipulated working hours. The final report of the National Commission for Enterprises in the Unorganized Sector (NCEUS) showed that the workers in the unorganized (informal) sector constitute more than 93 percent of the total workforce of India. The informal sector is characterized by minimum personal relationship between the employer and the employee. The most of the employees are contractual or casual. The labour laws are mostly, not applicable in the informal sector. There is lack of occupational safety & health awareness among them. Moreover, this tremendous work force directly links with work pressure. Work and time will become the stress to these workers. Production has great importance than safety and health, so, human comfort is greatly neglected. Demand for investigation on health and safety is a common and genuine demand of informal sectors. A major challenge is to come up with clear policies and actions aimed at supporting and nurturing the informal sector as well as improving health and safety in the sector. In a labour intensive country like India, Ergonomics has an immense application in different informal sectors in India. Until date, there have been very little consolidated efforts in informal sectors to enhance the productivity by the application of ergonomic health interventions. The intervention could be in the form of hand tool design. It could be also be in the area of workstation design in tandem with the user's anthropometric dimensions. Through

the participatory ergonomics approach, several interventions are designed and applied in different informal sectors of India. A detailed study is made on the identification of efficacies of these interventions. Surprisingly observed, low cost health interventions can improve the productivity of informal sectors up to 30%. The work-related musculoskeletal disorders (WMSDs) are also prevented. It is essential and important to apply the exact interventions and to find out and behavioural approaches of the users towards the utilizations of interventions. As informal sectors are increasing steadily in different developing countries so to give the workers comfort is becoming a challenge of these parts of the globe. The local authorities can begin by ensuring basic occupational health and safety among the informal sector workers so that they may realize their economic potential and can enhance organisational productivity.

Keywords: ergonomics, musculoskeletal disorders, productivity, intervention, safety

S6-2

An ergonomic intervention on the manual paddy threshing workstation for reducing occupational health problems of the workers

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In different states of India, threshing of paddy is done a manually operated threshing machine. The conventional manually operated threshing machine was used for threshing in standing posture. They used their right and left feet alternatively for pedaling to move the drum. There were some disadvantages for the workers while using conventional workstation from the viewpoint of human factors. The workers were required to bear the whole body weight on one leg (static load) during the operation for a long time while other leg was engaged in constant pedaling (dynamic load). As a consequence of this the leg muscle became fatigued and the workers reported pain in their leg. They were consistently required to adopt slightly forward bend posture for maintaining the 'body balance' during pedaling. Thus postural stress was imposed on the workers during using the conventional threshing machine. To resolve the said problems, ergonomic intervention was necessary to make the workstations became more user-friendly.

For the ergonomic intervention some steps were followed. The body part discomfort (BPD) rating and prevalence of musculoskeletal disorder of the workers was assessed while using the conventional workstation. After assessing the drawback a modified design concept was developed and accordingly some prototype model was made. Conversion of threshing workstation from a standing workstation to a sitting workstation was suggested for reducing high working stress on the legs of the workers. The seat height was optimized.

The results of the BPD rating revealed that the workers had a higher extent of pain or discomfort in the lower leg-calf and lower back than other segments of the body. The prevalence of MSD was also higher in those body segments than that of other body segments

The work surface height was determined on the basis of elbow rest height of the workers and it was 64.97 cm. The seat height was optimized by employing paired comparison test with different prototype models. The results showed that a height of 48 cm was the optimum for the users while using the threshing machine under sitting condition.

The modified workstation was again evaluated by employing different parameters, e.g. , Body part discomfort rating, working heart rate , EMG, back compression and productivity rate.

It was noted that ergonomic intervention reduced the discomfort level, physiological exertion and biomechanical stress of the workers during paddy threshing activity without affecting the productivity.

Keywords: paddy threshing, ergonomic intervention, posture, body part discomfort, biomechanics

S6-3

Workers of garment manufacturing in Kolkata: Increased susceptibility of inflammatory burst, COVID and non- communicàble diseases (NCDs)

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Textile industry including readymade garment manufacturing is the 2nd largest contributor of India in export. About 1lakh manufacturing units involving 3 million workers, mostly under informal sector are associated in this industry. Though, complaints of poor respiratory health of textile workers of different parts of India is common, no study has been performed on the workers of Garden Reach-Metiabruze area of Kolkata. In the 1st phase of this study, Pulmonary function test (PFT) by spirometry for Forced Vital Capacity, Forced Expiratory Volume1%, Slow Vital Capacity, Maximum Voluntary Ventilation and Peak Expiratory Flow Rate were evaluated. All these parameters were found to be significantly lower in the workers compared to control reflecting a mixed (obstructive & restrictive) with predominance of obstructive pattern of pulmonary function. Respiratory distresses like coughing, presence of phlegm, wheeziness, compression, chest tightness, breathlessness were also very high in them. Immediate recording of ambient environmental dust (PM_{10} and $PM_{2.5}$) unveiled that the work sites contained dust far beyond the permissible limit. Next, histological study and quantification of sputum macrophages were executed and the number of macrophages was higher than the people who were occupationally not exposed to dust. This primary stimulation of inflammatory lung response by over-production of alveolar macrophages may secondarily lead to a generalized rise in inflammatory response as evident by the rise in pro-inflammatory cytokines namely IL-1β, IL-6, γ-IFN & C-reactive protein levels in blood. Oxidative stress parameters like serum superoxide dismutase, catalase and plasma glutathione were low among the workers signifying a predominance of oxidative stress. Higher levels of pro-inflammatory cytokines may be associated with this augmented oxidative stress causing severe damage to various tissue systems leading to disease conditions including non- communicable diseases (NCDs). To explore the conditions of these workers in COVID era & post-COVID complications, a follow up survey was performed where more than 21% participants could not be traced out. Among the respondents, 90% reported about respiratory illnesses similar to COVID and a high proportion reported recurrence of the symptoms for 2/3 times within 2 years but very few were having officially diagnosed positive testing results. Among the non-respondents it was found that 6 workers were died of COVID -like respiratory infections. They also opined that the severity and frequency of respiratory tract infection has been increased after the COVID like infections. No concrete data on NCDs could be collected from them. Improvement in the working conditions and use of personal protection measures like masks can be beneficial in preventing the chronic inflammatory status of the workers. To achieve its goal of sustainable production, immediate assessment of health risks and their control measures are urgently warranted.

Keywords: informal textile workers, respiratory distress &sputum macrophages, proinflammatory cytokines, oxidative stress, COVID &infection susceptibility

S6-4

Musculoskeletal disorder and occupational stress in house maids of Kolkata, India

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Background: Work related musculoskeletal disorders (WMSDs) are very common among low income workers engaged in unorganised sectors due to their exposure to various stressful conditions. House maids perform varieties of household work and they globally constitute a significant percentage of unorganised sector workers. 94 female house maids of Kolkata (India) belonging to the age range of 20–60 years were recruited in this cross–sectional study to investigate the occupational stress index (OSI) and WMSDs among them. Evaluation of their morphological parameters, working condition, cardiovascular stress, working posture and body's centre of gravity (COG) were also attempted in the study.
Methods: Anthropometric and body composition parameters were evaluated by anthropometric rod and skinfold measurements, respectively. WMSDs and OSI were evaluated by relevant questionnaire methods while COG was measured by segmental method. Statistical Package for Social Sciences (SPSS) version 16.0 was used for the statistical analysis of the data.
Results: Body mass, body height, body surface area (BSA), waist and hip circumferences and lean body mass (LBM) depicted significant difference ($p<0.05$) in the age group of 41–50 years while waist–hip ratio (W/H Ratio) was significantly different in 51–60 years. The resting heart rate and blood pressure were within the normal range in all the age groups. Hand grip strength was lower in this population and showed significant ($p<0.05$) variation in different age groups. COG score varied in different working postures which were found to be unstable. OSI parameters depicted existence of excessive stress in this population and the OSI scores varied in different age groups. Assessment of WMSDs depicted different grades of pain in different parts of the body, maximum being the prevalence of low back pain. The intensity and localisation of pain varied in different age groups. Even, the organ wise pain discomfort varied during work, rest and sleep times.
Conclusion: Existence of high level of occupational stress may result in serious detrimental health hazards in this population. Urgent social attention is needed to attenuate this potentially high risk of physical and mental strain to sustain health. It is high time to develop effective stress management strategies not only to reduce their mental and physical stress but also to enable them to maintain healthy wellbeing.

Keywords: Informal work, Low back pain, Posture analysis, Occupational stress

S55-1

The roles of mitochondrial circFBXO25 in cardiac ischemia/reperfusion injury

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Background: Mitochondria can not only produce energy, but also regulate the life activities of cells by inducing apoptosis and necrosis, which is particularly important for heart disease. In fact, mitochondria can provide 90% ATP energy for cardiomyocytes, while the mitochondria pathway induced cell death is the main pathogenic factors of many cardiovascular diseases. In recent years, several studies have shown that circular RNAs (circRNAs) exist in mitochondria and plays an important role in regulating mitochondrial homeostasis. However, there are still few reports about nuclear-encoded circRNA which located in mitochondria, yet its function and transport mechanism are still unknown.
Methods: The circRNAs located in cardiac mitochondria were detected and identified by high-throughput sequencing, and then the expression of mitochondrial circFBXO25 were determined by qPCR assay in heart tissue and cardiomyocyte. The siRNA and adenovirus of circFBXO25 were used to respectively down-regulate or up-regulate the expression of circFBXO25. Besides, the cardiac cell death was detected by PI staining and LDH release, and the mitochondrial function was demonstrated by JC-1 staining, TMRM staining, Calcein-AM staining, and ATP content assay. In addition, the siRNA of TOM or PNPase were operated to detect the roles of TOM and PNPase in transporting circFBXO25 into mitochondria. Moreover, the Pulldown, mass assay and RIP assay were used to screen out and verified the ATP5PO as the targeting protein of circFBXO25. And the co-immunoprecipitation was operated to determine the interaction between ATP5PO and CypD.
Results: In this study, more than 3000 circRNAs located in mitochondria are found to be rich in the mitochondria of heart tissue. In addition, our results show that mitochondrial circFBXO25 encoded by nuclear genes is involved in the regulation of mitochondrial dysfunction and cardiomyocyte necrosis induced by hydrogen peroxide and ischemia/reperfusion respectively in vitro and in vivo. Moreover, we confirmed that circFBXO25 deficiency would further promote cardiac necrosis and mitochondrial dysfunction. Besides, the data also demonstrates that circFBXO25 overexpression protects cardiac mitochondria from oxidative stress by inhibiting the interaction between ATP5PO and CypD in vitro and in vivo. Additionally, our data demonstrate that Tom and PNPase are the essential regulators of entry of circFBXO25 into mitochondria.
Conclusion: The mitochondrial located circFBXO25 which is encoded by nuclear genome is a key positive regulator of protecting from cardiac oxidative stress injury, and TOM and PNPase regulate the process of circFBXO25 transporting into mitochondria. And circFBXO25 attenuates the cardiac injury through inhibiting the interaction between ATP5PO and CypD.

Keywords: mitochondrial circRNA, cardiovascular disease, ischemia/reperfusion injury, ATP5PO, transportation

S55-2

Interpretable - CEnter-Net: Interpretable hybrid model for automated multi-class classification of ECG signal based on the type of arrhythmia rhythms

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Background: Artificial Intelligence (AI) methods are highly employed in faster Electrocardiogram (ECG) rhythm interpretation with minimal manual errors possible in the diagnosis of different arrhythmia episodes. However due to the black box nature of AI, models are lacking the explainability (transparency) and interpretability of the results becoming difficult for users. Therefore, recently, Explainable AI (XAI) approaches are gaining popularity for making the AI models more explainable and interpretable.
Methods: In this paper, a new interpretable hybrid model, named

I-CEnter-Net (Interpretable-Convolutional Neural Network + Entropy + Classifier), is proposed for an automated classification of ECGs based on the type of arrhythmia rhythms present. Initially, normal and ECG signals having arrhythmia episodes are subjected to one dimensional Convolutional Neural network (CNN) to obtain deep features. These deep features are later concatenated with the handcrafted entropy features (Shannon Entropy (ShEn), Renyi Entropy (ReEn), Sample Entropy (SEn) and Approximate Entropy (ApEn)) extracted directly from ECG signals to form features vector. Finally, features vector is fed to Support Vector Machine (SVM) for automated multi-class classification of ECG signals as class0: sinus bradycardia, class 1: atrial flutter and atrial fibrillation, class 2: normal and class 3: ventricular trigeminy, ventricular flutter and ventricular tachycardia.
Results and Conclusion: The proposed CEnter-Net achieved an average accuracy of 98.5%, sensitivity of 99.32% and specificity of 97.60%. To make the proposed hybrid model's prediction interpretable, Local Interpretable Model-agnostic Explanations (LIME) method is employed which demonstrated visual explanation by highlighting the significant features responsible for and considered in model's decision making. Thus, the proposed ***I-CEnter-Net*** can aid the clinicians in faster identification of type of arrhythmia rhythms/episodes and in addition the significant features to investigate while assessing patient's ECG signals. Proposed system can be used as an adjunct tool in the clinical settings or as a screening tool in the polyclinics.

Keywords: arrhythmia, electrocardiogram, convolutional neural network, interpretability, LIME

S55-3

Empowering predictive precision with computational fluid dynamics in atherosclerosis prognosis

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The impact of human vascular hemodynamics on health and function is well-documented, with localized hemodynamic forces playing a crucial role in the progression of cardiovascular disease. Utilizing computational fluid dynamics (CFD) provides distinct advantages in diagnosing and analyzing cardiovascular conditions. Computational simulation of the vasculature provides accurate knowledge of experimentally unobtainable hemodynamic parameters in patients, including flow velocity, pressure, wall shear stress (WSS), and oscillatory shear stress under a variety of conditions. Notably, this approach, which starts with patient-specific imaging, facilitates a more realistic investigation of cardiovascular events.
In this study, we employed CFD analysis to assess pre- and post-atherosclerotic velocities and wall shear stress, predicting potential atherosclerotic stenosis lesions. Patient-specific 3D carotid and coronary artery geometries were reconstructed from computed tomography angiography images of both a control group and patients with ischemic heart disease. Simulation results showed that the atherosclerotic stenosis-prone sites received higher WSS, lower velocities, and distinct vortex patterns. The integration of WSS-based descriptors enables accurate prediction of atherosclerosis development locations. These predictions were subsequently validated using actual follow-up data from several patients. The proposed tool holds significant promise for real-world clinical scenarios, empowering clinicians to make informed decisions. For instance, the tool can aid in predicting prognosis for coronary intervention, suggesting optimal stent size or positioning.
In conclusion, our study underscores the potential of CFD analysis as a novel approach to predicting atherosclerotic lesions and associated cardiovascular events. The integration of patient-specific data and hemodynamic insights equips clinicians with a valuable tool for enhancing decision-making in clinical settings.

Keywords: computational fluid dynamics, wall shear stress, atherosclerosis, lesion prediction

S55-4

Genome editing based therapy for cardiovascular diseases

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Background: Pathogenic variants (PVs) in SCN5A can result in long QT syndrome type 3 (LQT3), a life-threatening genetic disease. Adenine base editors (ABEs) can convert targeted A•T base pairs to G•C base pairs, offering a promising tool to correct PVs.
Methods: We generated an LQT3 mouse model by introducing the T1307M PV into the *Scn5a* gene. The adenine base editor (ABEmax) was split into two smaller parts and delivered into the heart by adeno-associated virus serotype 9 (AAV9) to correct the T1307M PV.
Results: Both homozygous and heterozygous T1307M mice showed significant QT prolongation. Carbachol administration induced the torsades de pointes (TdP) or ventricular tachycardia (VT) for homozygous T1307M mice (20%) but not for heterozygous and wild-type (WT) mice. A single intraperitoneal injection of AAV9-ABEmax at postnatal day 14 resulted in up to 99.20% *Scn5a* transcripts corrected in T1307M mice. *Scn5a* mRNA correction rate of over 60% eliminated QT prolongation; *Scn5a* mRNA correction rate of less than 60% alleviated QT prolongation. Partial *Scn5a* correction resulted in cardiomyocyte heterogeneity, which did not induce severe arrhythmias. Furthermore, we did not detect off-target DNA and RNA editing events in ABEmax-treated mice hearts.
Conclusions: These findings show that *in vivo* AAV9-ABEmax editing can correct mutant *Scn5a* allele, effectively ameliorating arrhythmias phenotypes. Our results offer a proof-of-concept for the treatment of hereditary arrhythmias.

Keywords: gene therapy, arrhythmia, LQT3, base editor

S7-1

Reactivation of astrocytes reverses pain-like behaviour

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Astrocytes are the most abundant cell type in the central nervous system and play a key role in maintaining environmental homeostasis. However, recent studies have shown astrocytes also actively modulate neuronal circuit function, influencing processes such as synaptogenesis and synaptic plasticity. In mouse models of chronic pain, peripheral nerve injury triggers upregulation of astrocytic signaling pathways that affect dendritic spine physiology. In the somatosensory (S1) cortex, this causes maladaptive remodeling of pain and touch-related circuits by transiently promoting dendritic spine turnover, with these changes persisting even after astrocyte activity returns to baseline one week after injury. Therefore, we hypothesized astrocyte reactivation could be leveraged to induce therapeutic synaptic plasticity in S1 circuits as a novel therapy for chronic pain. We reasoned that activating S1 astrocytes whilst simultaneously blocking noxious afferent input to S1 would facilitate targeted disassembly of maladaptive noxious S1 circuits. To activate S1 astrocytes we applied transcranial direct current stimulation (tDCS at 0.1mA for 10 min) over the cortex of awake mice or used the Designer Receptors Exclusively Activated by Designer Drugs (DREADD) system (1.0 mg/kg clozapine N-oxide). Both approaches induced a sustained (hours) increase in the frequency and amplitude of Ca^{2+} transients in S1 astrocytes. To block noxious afferent input, we locally applied tetrodotoxin or lidocaine to the injured peripheral nerve via an implanted Elvax drug elution cuff or osmotic pump, respectively, with both treatments lasting for about five days. Using the von Frey behavioural assay and long term *in vivo* imaging of dendritic spines, we confirmed that this combined therapy permanently alleviated pain-related behaviors in mice which could be directly correlated with increased elimination of S1 dendritic spines. Overall, our study provides a proof of principle for astrocyte reactivation facilitating S1 circuit reorganization as a potential novel therapy for chronic pain.

Keywords: astrocyte, chronic pain

S7-2

Neuronal activity-dependent non-lethal caspase activity guides microglial synaptic phagocytosis and neuronal circuit remodeling

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Caspase activation in neurons has diverse outcomes, ranging from the regulation of neurogenesis and neurite remodeling to the initiation of cell death. Recent studies have suggested a link between local caspase activation at synapses and synaptic tagging by complements, which serves as an eat-me signal for microglial phagocytosis. However, the process triggering synaptic caspase activation and its involvement in synaptic phagocytosis by microglia remains unclear. Here, we found that neuronal activity-dependent non-apoptotic caspase activation at synapses promotes synaptic trogocytosis by microglia. Using a novel FRET system for real-time imaging of caspase3 activation, we discovered that increased neural activity induces localized and transient caspase3 activation at presynapses. Furthermore, optogenetic activation of caspase3 in pre-synapses induced synaptic tagging by complements. High-resolution real-time imaging revealed that complements selectively tag presynapses among neuronal compartments, allowing microglia to trogocytose presynapses without severing the axons. Lastly, experimental febrile seizures amplified caspase activity at inhibitory presynapses in the hippocampus, leading to complement-dependent microglial phagocytosis and resulting in increased seizure susceptibility. Our findings determine the cooperative role of caspases and complements in guiding microglial elimination of specific synapses, thereby influencing the remodeling of neuronal circuits.

Keywords: microglia, caspase, apoptosis, synapse, complement

S7-3

Glial control of thalamic sensory processing

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The representation and differentiation of sensory information in the brain remains a core question in neuroscience. While most studies on sensory processing have emphasized peripheral receptors or cortical function, the role of thalamic activity has been less explored. This underscores the need to investigate the mechanisms of thalamic sensory processing for a comprehensive understanding of sensory information processing in the brain. Recently, we discovered that astrocytes, a prevalent type of glia in the brain, synthesize and release tonic GABA in the thalamus. Notably, contrary to the prevailing belief that tonic GABA inhibits information processing, an elevation in tonic GABA within a physiologically relevant range amplified the temporal precision of neuronal responses in the thalamus, thereby enhancing tactile discrimination in mice. These findings offer novel evidence that astrocytes modulate tactile precision through tonic GABA in the thalamus. Based on our prior work and preliminary data, we propose that the glial network, comprising various cell types, is instrumental in mediating tonic GABA and modulating thalamic sensory processing, particularly by adjusting the synaptic integration and membrane characteristics of thalamocortical neurons.

Keywords: glia, thalamus, tonic gaba, sensory processing

S7-4

Astrocytic control of cognitive flexibility and conditioned place preference via tonic d-serine and glutamate release

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NMDA receptor (NMDAR) hypofunction has been implicated in several psychiatric disorders with impairment of cognitive flexibility. However, the molecular mechanism of how NMDAR hypofunction with decreased NMDAR tone causes the impairment of cognitive flexibility has been minimally understood. Furthermore, it has been unclear whether hippocampal astrocytes regulate NMDAR tone and cognitive flexibility. We employed cell type–specific genetic manipulations, ex vivo electrophysiological recordings, sniffer patch recordings, cutting-edge biosensor for norepinephrine, and behavioral assays to investigate whether astrocytes can regulate NMDAR tone by releasing D-serine and

glutamate. Subsequently, we further investigated the role of NMDAR tone in heterosynaptic long-term depression, metaplasticity, and cognitive flexibility. We found that hippocampal astrocytes regulate NMDAR tone via BEST1-mediated corelease of D-serine and glutamate. Best1 knockout mice exhibited reduced NMDAR tone and impairments of homosynaptic and a1 adrenergic receptor–dependent heterosynaptic long-term depression, which leads to defects in metaplasticity and cognitive flexibility. These impairments in Best1 knockout mice can be rescued by hippocampal astrocyte-specific BEST1 expression or enhanced NMDAR tone through D-serine supplement. D-serine injection in Best1 knockout mice during initial learning rescues subsequent reversal learning. These findings indicate that NMDAR tone during initial learning is important for subsequent learning, and hippocampal NMDAR tone regulated by astrocytic BEST1 is critical for heterosynaptic long-term depression, metaplasticity, and cognitive flexibility.
The underlying mechanisms of how positive emotional valence (e.g., pleasure) causes preference of an asso- ciated context is poorly understood. Here, we show that activation of astrocytic m-opioid receptor (MOR) drives conditioned place preference (CPP) by means of specific modulation of astrocytic MOR, an exemplar endogenous Gi protein-coupled receptor (Gi-GPCR), in the CA1 hippocampus. Long-term potentiation (LTP) induced by a subthreshold stimulation with the activation of astrocytic MOR at the Schaffer collateral pathway accounts for the memory acquisition to induce CPP. This astrocytic MOR-mediated LTP induction is dependent on astrocytic glutamate released upon activation of the astrocytic MOR and the consequent activation of the presynaptic mGluR1. The astrocytic MOR-dependent LTP and CPP were recapitulated by a chemogenetic activation of astrocyte-specifically expressed Gi-DREADD hM4Di. Our study reveals that the transduction of inhibitory Gi- signaling into augmented excitatory synaptic trans- mission through astrocytic glutamate is critical for the acquisition of contextual memory for CPP.

Keywords: TREK-1, Best1, channel-mediated release, astrocyte, synaptic plasticity

S7-5

Astrocytic Ca^{2+} prevents synaptic depotentiation in the motor cortex during motor learning

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Astrocytic Ca^{2+} signaling has been shown to regulate activity-dependent synaptic plasticity, but its role in learning-related synaptic changes in the living brain remains unknown. Here we show that motor training induced synaptic potentiation on apical dendrites of layer 5 pyramidal neurons, as well as robust Ca^{2+} elevation in the processes and somas of astrocytes in the mouse motor cortex. Reducing astrocytic Ca^{2+} by either the suppression of α1-IP_3R2 signaling or the activation of Gq-DREADD receptors in astrocytes led to synaptic depotentiation during motor learning and impairment of performance improvement. Notably, synaptic depotentiation occurred on a fraction of dendrites with repetitive dendritic Ca^{2+} activity. On these dendrites, spines active before dendritic Ca^{2+} activity underwent CaMKII-dependent synaptic depotentiation during motor learning. In addition, activating adenosine receptors prevented repetitive dendritic Ca^{2+} activity and synaptic depotentiation caused by the reduction of astrocytic Ca^{2+}, suggesting the involvement of ATP released from astrocytes and adenosine signaling in the process. Together, these findings reveal an important function of astrocytic Ca^{2+} in preventing synaptic depotentiation by limiting repetitive dendritic Ca^{2+} activity in the process of learning.

Keywords: astrocyte, synaptic plasticity, calcium, learning

S8-1

Oxidative stress as a unifying paradigm in the mosaic of hypertension

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Despite extensive research, the pathogenesis of hypertension is still elusive. Over 95% of patients have primary hypertension where the cause is unknown. However, what is clear is that the pathophysiology of primary hypertension is multifactorial, multifaceted, and highly complex and includes several interacting physiological systems. This is highlighted in the Page "mosaic theory" of hypertension where multiple interacting elements contribute to the pathophysiology of hypertension including genetic, environmental, and pathophysiologic factors that influence multiple systems including the vascular system. Hypertension is characteristically associated with vascular dysfunction, cardiovascular remodelling, renal dysfunction, and stimulation of the sympathetic nervous system. Emerging evidence indicates that the immune system is also important and that activated immune cells migrate and accumulate in tissues promoting inflammation, fibrosis, and target-organ damage. Common to these processes is oxidative stress, defined as an imbalance between oxidants and antioxidants in favour of the oxidants that leads to a disruption of oxidation-reduction (redox) signalling and control and molecular damage. Physiologically, reactive oxygen species (ROS) act as signalling molecules and influence cell function through highly regulated redox-sensitive signal transduction. In hypertension, oxidative stress promotes posttranslational modification (oxidation and phosphorylation) of proteins and aberrant signalling with consequent cell and tissue damage. Many enzymatic systems generate ROS, but NADPH oxidases (Nox) are the major sources in cells of the heart, vessels, kidneys, and immune system. Expression and activity of Noxs are increased in hypertension and are the major systems responsible for oxidative stress in cardiovascular disease. Here we provide a unifying concept where oxidative stress is a common mediator underlying pathophysiologic processes in hypertension.

Keywords: redox, blood pressure, hypertension, signaling, vascular biology

S8-2

Don't forget pregnant women and children

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One trend in cardiovascular medicine is to study the effects of drugs and interventions on women and not just men, to consider racial differences and the effects of age. We have appreciated for a long time that there are physiological differences associated with these characteristics, but it has taken a long time for the pharmaceutical and medical world to catch up. This was brought into highlight during the Covid pandemic when increased mortality with infection was found, in men vs women, non-white vs white, and old vs young.
Some groups however remain neglected – pregnant women and

babies. Most medicines given to pregnant women are untested in pregnancy. Of even more concern is, treatments for pregnancy specific conditions, such as threatened preterm birth, the vascular based pre-eclampsia, and dysfunctional labour, are often off-list use of drugs developed for other conditions. It remains the case that there is only one drug, syntocinon, synthetic oxytocin, to help women in dysfunctional labour, and it has low efficacy and high risk of harm, such as fetal hypoxia and uterine rupture. As well as most pharma being uninterested in helping pregnant women by developing new drugs, dating from the thalidomide scandal, the study of maternal and fetal physiology is being lost from many courses. I would like to see the future of cardiovascular and other areas of medicine, enriched by doing more for pregnant women and babies.

Keywords: pregnancy, oxytocin, preeclampsia, contractions, bloodflow

S8-3

Intracoronary hemodynamics for prevention of acute coronary syndrome or sudden cardiac death

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Invasive measurements of coronary artery blood flow and pressure can be used to assess whether atherosclerotic disease is causal of ischemia. Fractional flow reserve (FFR) is defined as the ratio of flow in the diseased vessel divided by the flow that would be attained in the vessel in the hypothetical case where the vessel was normal. FFR can be derived from pressure measurements assuming that the resistance to flow downstream of the measurement location would be the same in the hypothetical normal case. Non-invasive CT-derived computed FFR (CT-FFR) is a technology that enables non-invasive assessment of the functional significance of lesions from computational fluid dynamics (CFD) applied to coronary computed tomography angiography (CCTA). The scientific principles that underlie this technology are as follows. A three-dimensional patient specific anatomic model is first constructed from the CCTA data followed by physiological modeling. The basal coronary outlet resistances at rest are determined from myocardial mass and the vessel size at each outlet. The hyperemic condition is simulated by use of the experimental results that quantified the effect of adenosine. Finally, CFD analysis is performed to numerically solve the governing equations of fluid dynamics.

Since CT-FFR technology enabled non-invasive methods to model patient specific coronary geometry and physiology, this technology is applicable to plan the revascularization strategy and to the analysis of various hemodynamic parameters related with plaque progression and rupture. The CT-FFR solution encompasses pressure and velocity fields and thus wall shear stress (WSS) or traction can be easily derived for the quantification of total plaque force or stress. Non-invasive assessment of those hemodynamic parameters can provide valuable information on the possibility of plaque progression and on the identification of rupture prone plaques.

Plaque rupture can occur whenever plaque stress exceeds the plaque strength in a similar mechanism to general mechanical material failures. Therefore, if the imbalance between plaque durability and external force can be assessed together, this may improve discrimination of the risk of plaque rupture. Clinical application of non-invasive hemodynamic indices will provide additional insight in understanding the patient's vulnerability and its association with hemodynamic indices. In the EMERALD study, addition of non-invasive hemodynamics derived from CCTAT significantly improved the ability to predict the risk of ACS. The results were the same even in non-obstructive lesions. Ongoing EMERALD II study will validate this concept.

Keywords: coronary artery disease, coronary physiology, sudden death, acute coronary syndrome, coronary CT angiography

S8-4

Metabolic communication between cells in physiology and pathophysiology

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Cell metabolism generates energy equivalents and molecules required for cell division and growth. It is also now recognised that metabolites of different pathways can reprogram cells either via the modification of epigenetic marks or the post-translational modification of proteins. This presentation will highlight the link between metabolic pathways and cardiovascular disease playing special attention to acetylation, lactoylation and S-sulfhydration.

Keywords: post translational modification, metabolism, cardiovascular disease

S8-5

Nanostructural and functional remodelling underpinning the development of diabesity-linked heart failure; a breakdown in cellular quality control processes

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Obesity-related heart failure is a critically important global issue characterised by abnormal cardiac metabolic function leading to energy inefficiencies; however, the mechanisms and links to disease progression are incompletely understood. Employing 3-D volume electron microscopy techniques we investigated mitochondrial nanostructural properties in a murine model of early-stage type 2 diabetes (T2DM), exhibiting mild diastolic dysfunction, hyperglycemia and impaired OXPHOS. Structurally, mitochondrial subpopulations in the T2DM model, distinguished by the spatial distribution within the cardiomyocyte, are differentially affected compared to counterparts in the healthy myocardium; subsarcolemmal mitochondria (SSM) are enlarged (2-fold) and interfibrillar mitochondria (IFM) are more irregular in shape with tubular projections. Significantly, we have also characterised differential mitochondrial structural remodelling and a shift towards ßFAO in a mouse model of diet-induced obesity (DIO) (60% high fat diet, 12 weeks); suggesting a common response to diabesity-linked stresses. Mitochondrial pleomorphy is linked to adaptive alterations in mechanical load; however, the characterisation of disrupted cristae, coupled with our functional, molecular and proteomics data, supports the development of pathogenic remodelling. Another key finding is that mitochondrial density is increased in both models; in control cardiomyocytes mitochondria occupy 30.2 ± 1.1% of the cell volume whereas in the T2DM cardiomyocytes the occupancy is increased to 41.1 ± 2.7%; $P = 0.003$. In the obese heart mitochondrial density is similarly increased. We have now developed this work and identified a common underlying mechanism with novel links between Miro1-mediated mitophagy failure (part of the mitochondrial quality control processes) and arrested mitochondrial movement. In

conclusion, our studies show that cardiac mitochondrial dysfunction and aberrant structural modifications develop in obesity and mild T2DM, i.e. in the early stages of heart failure. A detailed understanding of the mechanistic basis of these remodelling events will have potential for identifying new targets to develop novel cardiometabolic therapies to prevent heart failure progression.

Keywords: heart failure, diabetic cardiomyopathy, mitochondria, mitophagy, structural biology

S9-1

Do-It-Yourself Physiology: Can the clinical application of Physiology be learnt online?

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Face-to-face laboratory classes have long been integral to physiology education (Zhang et al. 2021), allowing students to apply concepts, connect physiology with clinical practice and understand biological variation (Brinson 2015). However, as blended learning has become more prevalent, online laboratory lessons have supplemented or replaced face-to-face classes (Zhang et al. 2021). This study evaluated the efficacy of online physiology laboratory modules delivered during the COVID-19 pandemic. Participants were physiotherapy or speech pathology students (n=152) studying a second-year physiology course in 2020. Students experienced the first three weeks of semester face-to-face, and the remaining 10 weeks online. Face-to-face laboratory classes were converted into online modules incorporating video demonstrations, text, at home activities and formative questions. Students' use of laboratory modules was quantified using learning analytics. The results indicated that most students (55%) opened all seven laboratory modules, opening an average of 5.7 (SD 1.9) modules for 5.4 (SD 4.3) hours. The time spent on the modules correlated with students' laboratory examination marks (Pearson r = 0.33) and course performance (Pearson r = 0.34). However, the 2020 cohort did not perform as well as the previous cohort on the laboratory examination questions. Students' responses to an open-ended question regarding aspects of the course that helped or hindered learning were coded using inductive thematic analysis. Students most frequently cited the laboratory modules as helpful for learning (42%), although the lack of live laboratory classes was the most frequently cited aspect of the course that hindered learning (26%). Although students valued the online laboratory modules during the pandemic, these results indicate that some aspects of face-to-face laboratory classes may be difficult to replicate online. Specifically, laboratory classes provide students with opportunities to perform experiments and collect their own data in a collaborative learning environment. Therefore, online laboratory modules might better support learning as supplementary resources, rather than replacing face-to-face classes.

Zhang X, Al-Mekhled D and Choate J (2021). Are virtual physiology laboratories effective for student learning? A systematic review. *Adv Physiol Educ* 45: 467-480.

Brinson JR (2015). Learning outcome achievement in non-traditional (virtual and remote) versus traditional (hands-on) laboratories: A review of the empirical research. *Comp Educ Train* 87: 218–237.

Keywords: education, online learning, laboratory classes

S9-2

Progressive development of physiology knowledge and research skills using a blended learning approach

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The benefits of providing positive, constructive opportunities to develop students' knowledge of physiological concepts and research skills are widely recognized (Silverthorn, 2006). Covid-associated lockdowns required coordinators of biomedical science courses involving laboratory practicals to pivot rapidly to alternative, online practical experiences so students could still achieve intended course learning outcomes. This was undoubtedly a stressful time for both educators and students, and some immediate compromises in teaching quality were likely unavoidable. However, upon reflection, this major 'disruptor' has encouraged us to think more deeply about how we teach physiology laboratories and what we hope they accomplish (Choate et al., 2021).

Examples of curriculum delivery modifications triggered by pandemic-associated lockdowns that have persisted in the post-Covid lockdown era are presented. In a level 2 physiology course, in the face of Covid lockdowns, laboratory practicals were transitioned onto a cloud-based platform (Lt by AD Instruments) and students engaged with activities that checked and applied knowledge and guided them to analyze and interpret sample data. Workshops on research design, ethics and statistics were developed into online modules and students were supported within video conference-style sessions. Student Experience of Learning and Teaching (SELT) surveys revealed that some students were disappointed they could not participate in the on-campus version of laboratory practicals, primarily as they missed the social aspect of learning. However, many students also relayed appreciation that attempts had been made by course coordinators to retain integration and application of knowledge components.

In the Post-Covid lockdown era, emergency response online materials have evolved into pre-practical activities which students complete prior to attending on-campus laboratory sessions. Online workshops have been retained as they provide students with a consistent and equitable introduction to the topics whilst supporting progressive acquisition of research knowledge and skills. Retention of the modified approaches to teaching and learning have also led to positive outcomes for staff. Sessional teaching staff have fed-back that students arrive at laboratory practicals better prepared, with a clearer understanding of the rationale and specifics of what the lab sessions will involve. Practical facilitators and demonstrators can also work through online pre-practical activities and therefore have a richer understanding of concepts introduced to students. The blended approach of online materials and on campus activities has led to increased student satisfaction, with both students and staff feeling more comfortable with being prepared for on campus activities and have a better understanding of expectations on both them and the students they teach.

Silverthorn D. Adv Physiol Edu 30(4):135-40, 2006.

Choate et al. Adv Physiol Educ 45(2):310-321, 2021.

Keywords: blended learning, physiology practicals, research skills

S9-3

Beyond the pandemic: How will we use knowledge gained to aid student learning?

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Inquiry-based physiology laboratory classes provide valuable opportunities for students to reinforce their knowledge and develop skills in scientific methodologies and critical thinking (DiPasquale et al, 2003; Colthorpe et al, 2017). However, the COVID pandemic, rising costs and a desire for greater flexibility has led to a reduction in face-to-face (F2F) delivery of laboratory classes and an increasing use of online laboratory modules. Yet it is unclear whether the learning gains are equivalent between inquiry-based classes delivered in these differing teaching modes. This study evaluated students' perceptions of their learning from inquiry-based laboratory classes in alternate delivery modes: 'internal', delivered F2F or 'external', delivered via live zoom sessions. Biomedical science students enrolled in a 'Systems Physiology' course in internal (n=341) or external (n=117) modes undertook a series of inquiry-based laboratory classes. Students worked in groups to design and present an experiment proposal. Internal students undertook their experiment and analysed their data, whereas external students analysed data generated by prior students. All students completed an individual laboratory report using their analyses and evidence from the literature. Students were asked to identify their learning gains from these classes through open-ended questions. Responses were thematically analysed against an existing framework (Brinson 2015). Internal students reported gaining skills in data acquisition and presentation more than external students, whereas external students more frequently reported gaining skills in formulating aims and hypotheses. Students' grades on laboratory and course assessment items were identical in each mode. The outcomes of this study suggest that inquiry-based classes are readily adaptable to an online learning environment, with students equally able to develop many beneficial skills. However, students learning in a primarily online environment may need targeted support to assist development of specific skills. Potentially, a blended approach to inquiry-based laboratory classes may alleviate these issues while addressing student and institutional concerns regarding flexibility and budgetary constraints.

Brinson, JR. (2015) *Computers & Education*, 87, 218-237.
DiPasquale, DM., et al (2003) *Journal of College Science Teaching*, 32(6), 388-393.
Colthorpe, K., et al (2017) *Advances in Physiology Education*, 41(1), 154-162.

Keywords: inquiry-based laboratory, delivery mode, academic performance, education

S10-1

Physiology changes in muscle of elderly

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Human aging process is associated with progressive decline in neuromuscular function and performance which can cause disability and loss of independency. The process of myogenesis gradually deteriorates as the skeletal muscle ages, contributing to muscle mass loss.
Characteristics of skeletal muscle aging include reduction in myofiber plasticity, progressive loss of muscle mass and fast fibers, alteration in muscle-specific transcriptional mechanisms, and muscle atrophy.
Functional capacity degradation follows alterations in regenerative pathways of the functionality muscle tissues decrease in protein synthetic rates, increase in protein degradation, to affect biochemical, physiological, and morphological parameters of muscle fibers during the aging process.
Sarcopenia, although most specifically refers to loss of skeletal muscle mass, also includes progressive and generalized loss of muscle strength with a risk of adverse outcomes such as physical disability, poor quality of life and death.
Nowadays, sarcopenia is already accepted as one of geriatric syndromes, which are common, complex and costly states of impaired health in older individuals. Because the population of older individuals is increasing, the prevalence of sarcopenia is expected to be increase as well.
Sarcopenia can be categorized by its cause into primary (no other cause except ageing) or secondary (activity-related sarcopenia, disease-related sarcopenia and nutrition-related sarcopenia). Although the major contributor for sarcopenia is loss of skeletal muscle fibers because of decreasing motor neuron units, there are other factors that influence the development of sarcopenia such as: sedentary life style, physical inactivity, inadequate nutrition, malabsorption, cachexia, neurodegenerative diseases, and other age-related changes in hormonal status or cytokines. The mechanisms underlying sarcopenia include changes in muscle protein turnover, muscle remodeling, alfa motor neuron loss, muscle cells recruitment and apoptosis.
Sarcopenia has many bad implications such as precursor to nutritional frailty, drastic loss in body weight, blood glucose imbalance, impaired thermoregulation, development of chronic diseases, increases the risk of falls, reduced quality of life and increase in health care expenditures.
To improve economical and clinical consequences of sarcopenia, there are many therapeutic approaches that could be done such as hormonal intervention, nutritional and vitamin supplement, increase dietary protein intake, resistance training and pharmacological agents.

Keywords: muscle mass, muscle strength, sarcopenia, frailty

S10-2

Exercise, cellular senescence, and human longevity

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Background: To optimize healthspan in human adults, a lifelong strategy is required to shift the age profile of the cell population towards a younger direction while maintaining stable, functional weight. Recent intervention study has shown decreased all-cause mortality by increasing physical activity level for human adults aged > 50 y. How exercise as a catabolic stress improves survival fitness remains unclear.
Purpose: To address this question, we measure in vivo cellular senescence marker $p16^{INK4a}$ mRNA of replicable cells in human skeletal muscle following multiple muscle biopsy before and exercise.
Methods: $p16^{INK4a}$ expression is a robust biomarker of stem cell senescence in human tissues. Here we measured the effect of exercise intensity on in vivo senescence in skeletal muscle, using a randomized counter-balanced crossover trial. Biopsied vastus lateralis of 9 sedentary men (age 26.1±2.5 y) were assessed before and after a single bout of moderate steady state exercise (SSE, 60% maximal aerobic power) and high intensity interval exercise (HIIE, 120% maximal aerobic power) on a cycloergometer accumulating same amount of cycling work (in kilojoule).
Results: Increases in DNA strand break (+1.3 folds), and γ-H2AX+

myofibers (+1.1 folds) occurred immediately after HIIE and returned to baseline in 24 h ($p < 0.05$). Muscle p16INK4a mRNA decreased 24 h after HIIE (-57%, $p < 0.05$). SSE had no effect on DNA strand break and $p16^{INK4a}$ mRNA in muscle tissues. Senescence-lowering effect of HIIE was particularly prominent in the muscle with high pre-exercise $p16^{INK4a}$ expression, suggesting that exercise intensity determines the level of selection pressure to tissue stem cells at late senescent stage in human skeletal muscle. We have further found that NSAIDs delays the senolytic effect of exercise.
Conclusion: The anti-aging outcome appears to be due to a brief period of muscle inflammation during and after exercise, which may mediate the senolytic effect observed in human tissues.

Keywords: human skeletal muscle, senescence, senolytic, HIIT, NSAIDs

S10-3

Synergistic effects of exercise + milk product intake in older people: Background & evidence

Shizue Masuki

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Physical fitness peaks in our twenties; thereafter, it decreases by 5-10% every decade. After age 75, the rate accelerates, and when fitness has decreased to less than 30% of the peak level, people find it difficult to live independently. To prevent age-associated declines in physical fitness, we have developed a system of exercise training comprising interval walking training (IWT)** and an internet of things (IoT) system, which enables a large population to perform the training for a long time, at low cost, and with minimal personnel. Using this system, we examined the effects of 5-month IWT in more than 10,000 middle-aged and older people, and found that the training increased physical fitness by 15%, improved lifestyle-related disease (LSD) symptoms by 20%, and reduced healthcare costs by ~20% on average.
Regarding the mechanism for the improvement of LSD symptoms, increased physical fitness improved mitochondrial function, which, in turn, suppressed chronic systemic inflammation. Experimentally, we found that the methylation of pro-inflammatory gene, *NFKB2*, was enhanced by the training.
Next, we examined any merits of nutritional supplements when they were consumed during IWT. We found that milk product intake during IWT enhanced methylation of *NFKB* genes and other pro-inflammatory cytokine genes in conjunction with an increase in thigh muscle strength, which was accompanied by increased carotid arterial compliance and improved blood glucose control function.
Recently, we have also developed a mobile application program to provide participants with this service on their smartphones so that we can examine the effects of IWT in a much larger population of people at the same time and across generations. The system has great potential to increase interdisciplinary studies between exercise physiology and other fields in order to establish and promote a society for health and longevity.
**IWT: Walking training regimen to repeat slow and fast walking at ~40% and >70% of individual peak aerobic capacity, respectively, for 3 min each per set, >5 sets per day, and >4 days/wk.

Keywords: interval walking training, physical fitness, chronic inflammation, exercise intensity, IoT system

S10-4

The impact of community exercise programmes on metabolic health and muscle functions in older men and women

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Current evidence supporting the effects of exercise on muscular and metabolic health are derived primarily from programmes that are execute in controlled experimental settings. The experimental approach may not accurately reflect the exercise behaviour of elderly in the natural community settings, where participation is usually self-directed and limited to one mode of exercise, with varied exercise volumes, depending on personal, social, and environmental factors. The effects of community exercise participation or structured programs in community settings on metabolic and muscular health and functions of elderly people are not well-studied. Therefore, this presentation aims to discuss results from our studies that investigated the effects of exercise on metabolic and muscular health in young to older men and women in community settings. Although active lifestyle can lower the risk of glucose dysregulation in young and older individuals, the difference in glycemic risk was demonstrated by plasma insulin response, and not by blood glucose concentration. In healthy sedentary individuals, the poorer rate of glucose absorption can be compensated by higher insulin secretion to regulate blood glucose within normal range. This finding has significant implications on the use of fasted blood glucose to assess glycemic risk in healthy individuals and supports the switch to plasma insulin for assessment of glycemic risk before the onset of disease. Our results also showed that the volume of exercise performed weekly is as important as the mode of exercise in preserving muscle health and functions in older individuals. The mode of exercise performed has greater influence on muscle functions than overall MSK mass. These results support the current evidence and international guidelines advocating the use of exercise as a core public health intervention as a multi-potent "pill" to prevent and treat common chronic disease.

Keywords: Ageing, Metabolic health, muscle functions, exercise

S10-5

Core strength training to improve strength and flexibility of older people

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Introduction: The core strength is fundamental for older people to engage in physical activity. The "core" consists of the lumbar spine, pelvis, hip joints, and structures that control movement throughout this body area. Core area muscles include the transversus abdominis, multifidus, diaphragm, and pelvis. It will be floor muscles. They work together to provide stability to the spine.
Aims: The study investigated the effect of core strength training for 16 weeks to improve strength and flexibility in older men and women.
Methods: Forty elderly men and women aged (60-90 years old) in Panti Wredha Pucang Gading Semarang were subject to the study. The study conducts a quasi-experimental pre-posttest design—sixteen weeks of Core Strength Training (CST) intervention for older people. Before and after the CST intervention, the core strength and flexibility tests were tested.
Results: The intervention study showed a significant effect of the CST

test compared to the pre and post-test ($p < 0.05$). The flexibility test showed no significant comparison before and after the intervention of CST ($p > 0.05$).
Conclusion: Core strength training improves the strength of older people. Even though there is no effect on flexibility, the training can support seniors in doing easy exercises to strengthen the core muscle.

Keywords: elderly, strength, flexibility, exercise, core

S11-1

Unilateral ephaptic program for sweetness dominance

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Taste perception represents interactions between primary taste qualities, where sweetness predominates by inhibiting other tastes in humans, favoring carbohydrate ingestion. Meanwhile, sweet-induced gustatory suppression must not compromise individual responsiveness of aversive tastes required for animals to survive undesirable food. However, it is unknown how gustatory processing achieves sweetness dominance concurrently supporting the sensitivity despite the suppressibility. Here, we discover that, expressed in sweet-sensing gustatory receptor neurons (sGRNs), the hyperpolarization-activated cyclic nucleotide-gated channel (HCN) encoded by *Drosophila Ih* underlies sweet-dependent suppression, allowing facilitated feeding responses to bitter-laced sweets. Sweet-induced bGRN inhibition requires excitation of, but not synaptic transmission from sGRNs, indicating ephaptic coupling between GRNs. Unlike previously characterized olfactory receptor neurons, GRN coupling is unidirectional due to HCN expressed in sGRNs that resists bGRN-provoked inhibition, averting bidirectionality-caused signal dampening. Provided the adoption of HCN as a directional determinant, ephaptic signaling may have been deliberately evolved as a genuine device of information processing rather than being coincidental electrical interference during excitations of neighboring neurons.

Keywords: taste interaction, sweetness dominance, ephapic coupling, Drosophila, feeding

S11-2

Decoding the molecular logic underlying the evolution of vertebrate locomotion

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Walking is one of the major forms of locomotion displayed by land vertebrates, but the origin of neuronal circuit for limb control has not been well understood. Previously, we showed that the little skate Leucoraja erinacea, a cartilaginous fish, displays a pelvic fin driven walking-like behavior using genetic programs and neuronal subtypes similar to those of land vertebrates, suggesting preexisting neuronal circuits and genetic programs of common ancestor of little skate and tetrapods evolved through adaptation in all vertebrates with paired appendages.
However, mechanistic studies on little skate motor circuit development have been limited, due to a lack of high-quality reference genome. Here, we generated an assembly of the little skate genome, with precise gene annotation and structures, which allowed post-genome analysis of spinal motor neurons (MNs) essential for locomotion. Through interspecies comparison of mouse, skate and chicken MN transcriptomes, shared and divergent gene expression profiles were identified. Comparison of accessible chromatin regions between mouse and skate MNs predicted shared transcription factor (TF) motifs with divergent ones, which could be used for achieving differential regulation of MN-expressed genes. In accordance with the difference in the complexity of locomotion displayed by these two species, a greater number of TF motif predictions were observed in MN-expressed genes in mouse than in little skate. These findings suggest conserved and divergent molecular mechanisms controlling MN development of vertebrates during evolution, which might contribute to intricate gene regulatory networks in the emergence of a more sophisticated motor system in tetrapods.

Keywords: locomotion, evolution, genome, little skate, motor neuron

S11-3

Maternal aging affects the activities of a sensory neuron via a small RNA pathway

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Small RNAs regulate gene expression and modulate animal behavior. However, the roles and mechanisms of small RNAs in animal behaviors still need to be understood. Here, we show that the small RNA pathway regulates the neuronal activities of a sensory neuron-type through the neuropeptide gene expression in an interneuron. *C. elegans* ascr#3 (asc-ΔC9, C9) pheromone elicits avoidance behavior in wild-type hermaphrodites (Jang et al., 2012). We found that the exoribonuclease *eri-1* is necessary and sufficient for ascr#3 avoidance behavior; *eri-1* mutants and over-expressed animals exhibit decreased and increased ascr#3 avoidance, respectively. *eri-1* acts in the AVH interneurons to promote the mRNA level of the FMRFamide-like gene *flp-26 in AVH*, *which* is required for the sensitivity of the ADL-pheromone sensing neurons to ascr#3. We then identified a microRNA *mir-8207* as an *eri-1* target that directly regulates *flp-26* mRNA levels in AVH. Interestingly, ascr#3 avoidance behavior of offspring is decreased with the mother's age that regulates *eri-1* expression in the AVH neurons of progeny. Our study provides a circuit mechanism underlying maternal aging-mediated behavioral plasticity and helps to understand the roles of small RNAs in animal behavior at the circuit level.

Keywords: maternal aging, C. elegans, behavioral plasticity

S11-4

Aversive memory induced by mitochondrial stress: A *C. elegans* model

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Aversive memory allows the animals to avoid potential hazards and increases their chance of survival. The nervous system associates cellular stress with causative or concurrent external cues to form aversive associative memory, which drives avoidance behavior to minimize

exposure to those sensory cues. Important unanswered questions include the neural circuits that underlie aversive memory, the genes that encode the memory signals, and the neuronal activity patterns that represent memory formation or recall. We recently established an invertebrate model of mitochondrial stress-induced aversive memory, using the genetically tractable roundworm *Caenorhabditis elegans*. We identified critical genes and neuromodulators that trigger memory formation under mitochondrial stress; we also mapped several neuronal circuits that drive such aversive memory. In addition, we characterized unique neuronal dynamics patterns that represent synaptic plasticity for early memory traces. Our work has revealed important insights for the genetic and neural mechanisms of memory under physiological stress.

Keywords: aversive memory, mitochondria, C. elegans, neural circuit, neuromodulation

S12-1

Microbial metabolites and nutrient-sensing signaling in central neural regulation of blood pressure

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Maternal nutrition influences the composition of fetal microbiota, hence health of the offspring. In a rodent model of hypertension programmed by maternal high fructose diet (HFD) exposure, we found that levels of the microbiota-derived metabolites, particularly butyrate and propionate of the short-chain fatty acids (SCFAs), were significantly reduced in the plasma of adult offspring. The decreased tissue level of butyrate and increased expression of SCFA-sensing receptors, GPR41 and olfr78, in the hypothalamic paraventricular nucleus (PVN) of HFD offspring were rectified by oral supplement with –biotics (prebiotic, probiotic, and synbiotic) to young HFD offspring. Oral supplement with postbiotic butyrate restored tissue butyrate levels and rectified expressions of GPR41 and olfr78 in PVN, whilst propionate treatment mainly influenced gut microbiota composition and altered the abundance of genera Anaerovorax, Lactobacillus, Macellibacteroides, and Rothia. Both treatments notably protected against programmed hypertension in adult HFD offspring. In the rostral ventrolateral medulla (RVLM), where sympathetic premotor neurons reside, the nutrient-sensing signaling of AMP-activated protein kinase (AMPK)-sirtuin1-peroxisome proliferator-activated receptor γ co-activator α (PGC-1α) signaling for mitochondrial biogenesis and redox homeostasis were significantly impaired in young HFD offspring. Oral administration of a HMG-CoA reductase inhibitor, simvastatin, or an AMPK activator, metformin, to young HFD offspring preserved mitochondrial biogenesis, alleviated the production of reactive oxygen species in RVLM, and attenuated sympathoexcitation and programmed hypertension. These data suggest that dysfunction of AMPK-SIRT1-PGC1α nutrient sensing signaling may contribute to tissue oxidative stress in RVLM, which in turn primes increases of sympathetic vasomotor activity and blood pressure in young offspring programmed by excessive maternal fructose consumption. At the same time, alterations in tissue butyrate level and expression of GPR41 and olfr78 in PVN could be novel mechanisms that underlie hypertension programmed by maternal HFD exposure in adult offspring. Furthermore, oral –biotics supplementation may exert beneficial effects on hypertension of developmental origin by targeting dysfunctional SCFA-sensing receptors in the PVN.

Keywords: short-chain fatty acid, nutrient sensing signaling, microbiota, programmed hypertension

S12-2

Molecular pathway of exercise-induced health in mouse model

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Microbiota is an important enhancer of exercise performance and is a regulator of host physiology and energy metabolism through beneficial metabolite production by bacterial fermentation. In this study, we discovered that germ-free (GF) mice had a reduced capability for aerobic exercise as well as low oxygen consumption rates and glucose availability. Surprisingly, GF mice showed lower body weight gain and lower fat mass than specific pathogen-free (SPF) mice. Therefore, we hypothesized that these paradoxical phenotypes could be mediated by a compensatory increase in lipolysis in adipose tissues owing to impaired glucose utilization in the skeletal muscle. Our data revealed that gut microbiota depletion impairs host aerobic exercise capacity via the deterioration of glucose storage and utilization. The improved browning ability of GF mice may have contributed to the lean phenotype and could have negatively affected energy generation. These adaptations limit obesity in GF mice, but impede their immediate fuel supply during exercise, resulting in lower exercise performance.

Keywords: germ-free, microbiota, exercise, glucose metabolism, skeletal muscle

S12-3

Transcriptional and signal transduction underlying neuromodulation of heart rhythm in exercise and disease

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Background: Exercise training reduces oxidative stress and causes marked alterations in cardiac sympathovagal balance. Athletes have reduced sympathetic activation during exercise and enhanced vagal responsiveness during recovery compared to sedentary controls. Conversely, the opposite autonomic phenotype is a hallmark of many primary cardiovascular diseases, and is a negative prognostic indicator for morbidity and mortality associated with arrhythmia and sudden death. We have tested the idea that exercise training reduces oxidative stress and enhances the nitric oxide cGMP pathway to improve sympathovagal function, whereas impairment of this pathway results in cardiac dysautonomia and poor cardiovascular outcomes.
Method: Mice undertook 10 weeks voluntary wheel-running (+EX, *n*= 27; peaked 9.8 ± 0.6 km day^{-1} at 5 weeks), whereas control mice were housed in cages without wheels (-EX, *n*= 27). Guinea-pigs underwent 6 weeks of swim training (+EX n=20, -EX n=20). In both species, tissue was removed and assessed for adaptation to exercise training, molecular analysis of the NO-cGMP pathway, and the autonomic regulation of cardiac excitability using a neural-atria preparation *in vitro*. Gene transfer of neuronal NOS was used to probe the utility of sympathovagal responsiveness following exercise training and in diseased states.
Results: Exercise training caused adaptations of increased ventricular weight/body weight ratio, enhanced skeletal muscle citrate synthase activity and higher concentrations of [^{3}H]ouabain binding sites in both

skeletal and cardiac tissue. Training also increased nNOS protein in both species, whereas it was decreased in post MI Guinea-pigs and hypertensive rats. Exercise trained mice had an increase in cardiac vagal responsiveness and trained guinea-pigs had a decreased sympathetic responsiveness. Both responses were modulated by NOS inhibition. nNOS knockout mice failed to generate a vagal phenotype even though they maintained exercise performance. Gene transfer of nNOS into the intracardiac ganglia restored vagal function in mutant mice and also in guinea-pigs with impaired vagal neurotransmission following MI. Moreover, gene transfer of nNOS into stellate neurons mimicked the attenuated sympathetic response elicited by exercise training, and also decreased sympathetic hyperactivity in hypertensive rats.
Conclusions: nNOS appears to be a key protein in generating the cardiac sympathovagal phenotype elicited by exercise training. Gene transfer of nNOS into cardiac autonomic neurons may provide therapeutic utility to restore neural balance in states of dysautonomia caused by cardiovascular disease where the NOS-cGMP pathways is also impaired.

Keywords: nitric oxide, gene transfer, heart, sympathetic nervous system, vagus

S12-4

Roles of mitochondrial Ca^{2+} dynamics during cardiac workload transition

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Mitochondrial Ca^{2+} is an important factor regulating energy metabolism, cell death pathway, cytosolic Ca^{2+} signaling, and so on. It is not static, but changes dynamically. In cardiac mitochondria, Ca^{2+} uniporter MCU and Na^{+}-Ca^{2+} exchanger NCLX have dominant roles in Ca^{2+} influx and efflux, respectively. We previously reported that the NCLX activity is closely associated with sarcoplasmic reticulum (SR) Ca^{2+} dynamics. In order to get more insights into the mitochondria-SR interactions and the physiological roles, we carried out a "physiome" study, a combination of experiments and model analyses.
Bimolecular fluorescence complementation assays demonstrated that the exogenously expressed NCLX was localized in close proximity to exogenously expressed SR Ca^{2+} pump SERCA1-3 in HEK293 cells. Immunofluorescence analyses of isolated mouse cardiac mitochondria and isolated mouse ventricular myocytes visualized that the endogenous NCLX was localized near SR proteins, SERCA2 and ryanodine receptor RyR. Super-resolution imaging analyses revealed that co-localization coefficients were higher for the NCLX-SERCA2 than for the NCLX-RyR, confirming the close localization of endogenous NCLX and SERCA2 in cardiomyocytes. In HL-1 cardiomyocytes, a cell line derived from mouse atrial myocytes, pharmacological inhibition and knockdown of NCLX resulted in the decreased SR Ca^{2+} content, the slowed SR Ca^{2+} reuptake, and the slowed firing rate.
Mathematical cardiac cell models implemented with the spatial and functional coupling of NCLX and SERCA well reproduced the NCLX inhibition-mediated modulations of SR Ca^{2+} dynamics. We will discuss the roles of the coupling in response to the workload transition.
References. 1. Takeuchi et al., Sci Rep, 2013; 2. Takeuchi and Matsuoka, Int J Mol Sci, 2022.

Keywords: cardiomyocyte, mitochondria, sarcoplasmic reticulum, calcium, physiome

S12-5

The athlete's heart and ARVC: When the desmosomal reserve is not good enough

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Plakophilin-2 (PKP2) is a protein of the desmosome, an intercellular adhesion structure. Mutations in the gene coding for PKP2 associate with most cases of gene-positive arrhythmogenic right ventricular cardiomyopathy (ARVC), a disease characterized by the loss of muscle mass at the expense of fibrofatty infiltrates (predominantly in the right ventricle), ventricular arrhythmias, and high propensity for sudden death in the young. Importantly, exercise in desmosomal mutation carriers (including PKP2) significantly increases the risk of developing the cardiomyopathy, accelerates the progression to heart failure, and increases the occurrence of arrhythmias and sudden death. Current ARVC therapy is not curative, and only mildly effective in alleviating symptoms and containing disease progression. The long-term goals of my laboratory are to advance our understanding of ARVC molecular mechanisms, and to generate pre-clinical knowledge that can improve ARVC therapy and evaluation of risk. In this seminar I will present our latest findings on the molecular changes that lead to exercise-induced arrhythmogenic cardiomyopathy, and current studies aiming to develop safe and effective strategies for medical treatment of the affected population.

Keywords: arrhythmogenic cardiomyopathy, right ventricle, exercise, Plakophilin-2, desmosomal reserve

S40-1

Intersection of trained immunity and metabolism in macrophages through SETDB2

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Several pro-inflammatory signals such as microbial cell derived β-glucan, have been identified as initial "triggers" that train macrophages to respond to a later secondary challenge. This relatively crude memory response is mediated at least in part through epigenomic changes to the chromatin landscape. SETDB2 is a putative epigenomic modifying enzyme and is a member of the KMT1 family of lysine methyl-transferases including GLP1 and G9A.
To explore Setdb2 in innate immune responses, we first compared bone marrow derived cells from WT and myeloid deficient Setdb2 (Setdb2mKO) in a two-step treatment protocol. Cells were plated and "trained" with β-glucan for 24 hr. and following six days in culture with GMCSF cells were challenged with LPS. In the absence of glucan treatment, LPS challenge stimulated proinflammatory genes and lactate production similarly in WT and Setdb2mKO. However, the robust β-glucan dependent enhancement of LPS response observed in WT was lost in Setdb2mKO. Next, we performed RNA-sequencing in WT vs Setdb2mKO and results showed ~2200 differentially regulated genes between WT vs. Setdb2mKO BMDMs. Further *k-means* clustering revealed a dynamically changing profile of clustered transcripts with 5 unique patterns. Cluster 1 (475 genes) exhibited a positive correlation with Setdb2; they were super-induced by β-glucan training in WT and the effect was blunted in Setdb2mKO. In contrast, cluster 2 (857 genes) correlated negatively with SETDB2 in response to training; LPS

dependent activation observed in WT was suppressed by β-glucan pre-training and suppression was blocked in the Setdb2mKO. Pathway analysis revealed Cluster 1 genes are associated with NFkB signaling, hypoxia, and glycolysis whereas Cluster 2 genes are enriched for interferon gamma and alpha regulated inflammatory pathways. The responses are consistent with Setdb2 activating genes in Cluster 1 and inhibiting target genes in Cluster 2.
We generated a knock-in mouse line with two amino acid substitutions that convert two key residues involved in coordinating the binding of SAM to alanines (Setdb2KI), which would abolish any methyltransferase activity. Interestingly, unlike in Setdb2mKO, super induction by LPS following β-glucan training was maintained in BMDM from the Setdb2KI. In contrast, Interferon responsive genes from Cluster 2 that were negatively regulated by β-glucan in WT BMDMs but not in the Setdb2mKO, were also not suppressed in BMDMs from the Setdb2KI mice. These results provide an explanation for seemingly paradoxical results in literature and suggest Setdb2 may regulate different immune response pathways by two different molecular mechanisms; one associated with gene repression and may require enzyme activity and the other, which does not require Setdb2 enzyme activity but instead is mediated through chromatin looping. We are currently testing these two predictions.

Keywords: SETDB2, epigenomics, immune training, macrophages, inflammation

S40-2

Cold-signal-sensing histone demethylase regulates brown and beige adipocyte activation by distinct mechanisms to prevent obesity

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Lifestyle diseases such as type 2 diabetes, which are often associated with obesity, can be caused by both genetic and environmental factors. Environmental cues can regulate gene expression through various pathway, including signaling and transcriptional pathway as well as epigenetic modifications such as DNA methylation and histone acetylation and methylation. Histone modifications such as di- and tri-methylation of the 9th lysine of the histone 3 can repress transcription, but a histone demethylase called JMJD1A removes these modifications to activate gene expression. Mice with a JMJD1A gene deletion have been found to exhibit metabolic disorders such as obesity, insulin resistance, impaired glucose tolerance, and dyslipidemia.
We have discovered that JMJD1A is regulated by the beta-adrenergic signal, which phosphorylates serine residue 265 (S265) of JMJD1A through protein kinase A when the sympathetic nerve is activated in response to cold environments (signal sensing) and controls its function (Abe et al *Nat Commun* 2015, Inagaki et al *Nat Rev. Mol Cell Biol* 2016). This activation leads to the formation of protein complex between phosphorylated JMJD1A and chromatin remodeling factors and nuclear receptors in brown adipose tissue (BAT), promoting rapid expression of thermogenic genes through changes in higher order chromatin structures. In chronic cold environments, white adipose tissue activates quiescent thermogenic genes via histone demethylation, inducing them to become beige adipocytes with thermogenic function. Mice with a mutation of histidine residues in the demethylation activation domain to tyrosine show reduced browning of white adipose tissue, resulting in obesity, impaired glucose tolerance, insulin resistance and dyslipidemia, along with chronic inflammation in adipose tissue.
In contrast, mice with an alanine mutation in the S265 signal sensing site exhibit reduced activation of brown adipocytes during acute cold exposure, low body temperature, and suppressed browning of white adipose tissue (WAT) under chronic cold exposure (Abe et al *Nat Commun* 2018).
To promote browning, we investigated if the activity of JMJD1A could be increased. We found that the dephosphatase MYPT1/PP1β complex inhibits phosphorylated JMJD1A, and its absence in adipose tissue-specific knockout mice led to improved cold tolerance, reduced weight gain on a high-fat diet, and improved glucose tolerance and hyperinsulinemia. In my talk, I will discuss the role of MYPT1 in obesity through the "environmental stress-signal-epigenomic regulatory axis" that we have uncovered based on our research findings. (Takahashi H, *Nat Commun* 2022, Matsumura et al *Nat Metab* 2023).

Keywords: epigenetics, coactivator, beige adipocyte, histone demethylase, obesity

S40-3

Adipose tissue plasticity and energy metabolism

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White adipose tissue (WAT) is a central organ of lipid and glucose metabolism that impacts systemic energy homeostasis. WAT actively senses nutritional changes and accordingly stores extra energy in the form of triglycerides or supplies nutrients to other organs. Also, WAT regulates whole-body energy metabolism by communicating locally and with distant tissues by secreting signaling molecules such as adipokines, lipokines, metabolites, and exosomes. In obesity, multiple insults promote aberrant gene expression in WAT and lead to WAT dysfunction. Obesity is one of the major risk factors for the development of metabolic diseases such as cardiovascular diseases, type 2 diabetes, and atherosclerosis as well as cancer. One hallmark of obesity is the extensive expansion of WAT that is characterized by maladaptive remodeling events including increased adipocyte hypertrophy, impaired formation of new adipocytes, and accumulation of pro-inflammatory immune cells. Accordingly, significant advances have been made over the past few decades in the understanding of obesity-induced aberrant WAT remodeling and its pathophysiology with respect to obesity-related metabolic disorders. In this presentation, I will discuss the heterogeneity of adipose tissue and energy homeostasis with crosstalk between multiple cell types.

Keywords: adipose tissue, gene expression, heterogeneity, obesity, single cell analysis

S40-4

Role of CRTC2 in metabolic derangement

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The white adipose tissues (WAT) are crucial in maintaining metabolic homeostasis in mammals. Deterioration functional WAT is causal to the age-associated metabolic disorders, although the role of mature adipocytes in this process has yet to be defined. Here we explored the

role of adipose CRTC2 in the instigation of metabolic derangement in response to aging. Age-associated increase of CRTC2 in WAT led to the enhanced mTORC1 pathway via a reduced BCAA catabolism, which was abrogated by adipocyte-specific depletion of CRTC2. Mechanistically, CRTC2 functions as a transcriptional regulator of HES-1, leading to the reduction in BCAA catabolism and the increased incidence of cellular senescence. By single-cell RNA sequencing analysis, we found that increased CRTC2 activity in mature adipocytes led to the age-associated remodeling of WAT architecture by promoting the secretion of senescence associated secretion phenotype (SASP) factors. These data collectively suggest that adipose CRTC2 is critical in the age-associated metabolic decline by promoting cellular senescence in a paracrine manner.

Keywords: white adipose tissues, aging, metabolic disorder, CRTC2, senescence

S13-1

Cellular senescence as a therapeutic target for age-related disorders

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Evidence indicates the pathogenic role of cellular senescence in age-related cardiovascular-metabolic disorders including heart failure, atherosclerotic diseases, obesity, and diabetes. Protein p53 is described as a "guardian of the genome", but is also known to mediate cellular senescence. Activation of p53 was observed in aged vessels, and failing hearts, and this is recognized to promote pathogenesis in atherosclerosis or heart failure. Suppression of cellular senescence takes the risk of tumorigenesis, and more safe approach needs to be explored to suppress the accumulation of senescent cells. Recently, the senolytic approach opened a new avenue for aging research. Senolysis, the specific depletion of senescent cells, mediated through the genetic/ pharmacologic/ vaccination approach reversed aging and pathologies in age-related diseases. Specific depletion of senescent cells would become next-generation therapies for cardiovascular diseases.

Keywords: senolysis, cell senescence

S13-2

Clinical benefits of fenofibrate in obesity-related heart failure and its cardiac mechanisms

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Heart failure (HF) is a common and serious condition that affects millions of people worldwide. Fenofibrate, a peroxisome proliferator-activated receptor alpha (PPARα) agonist, favorably modulates lipid metabolism and avoids insulin resistance. Although, obesity is the major cause of metabolic disorders including diabetes and cardiovascular disease (CVD), there is still more to learn about the protective role of PPARα in obesity-related cardiomyopathy. Here, we investigated the effects of fenofibrate on HF requiring hospital administration (advanced HF) using the Korean National Health Insurance database from 2010 to 2017. Among participants who had already used statins, 427,154 fenofibrate users were identified and compared with 427,154 fenofibrate non-users with 1:1 age- and sex-adjusted matching. During 4.22-year follow-up, fenofibrate use significantly reduced the risk of advanced HF requiring hospital admission (hazard ratio (HR), 0.907; 95% CI 0.824-0.998). In subgroup analysis, fenofibrate use showed significantly reduced the risk of HF requiring hospital admission in obese group (HR, 0.866; 95% CI 0.762-0.985). Intriguingly, HR of fenofibrate use for advanced HF was lowest in chronic kidney disease group (HR, 0.769; 95% CI 0.644-0.919). Then we explored underlying mechanism of beneficial effects of PPARα activation using db/db mice as a model of obesity-related cardiomyopathy to examine how fenofibrate impacts heart function. After 8 weeks of fenofibrate treatment, db/db mice treated with fenofibrate showed lower weight gain, improved glucose homeostasis, and lower triglyceride levels in the blood compared to vehicle-treated db/db mice. Histological analysis showed that fenofibrate treatment reduced fibrosis and lipid accumulation. Transcriptome analysis has shown that fenofibrate suppresses inflammatory and immunological responses in the heart via TNF (tumor necrosis factor) signaling. Our findings suggest that fenofibrate can be used as a therapeutic agent for obesity-related cardiomyopathy.

Keywords: diabetic cardiomyopathy (DCM), peroxisome proliferator-activated receptor (PPAR)-alpha, fenofibrate, lipid metabolism

S13-3

Targeting the inflammation-driven cell phenotypic changes for cardiovascular therapy

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Inflammation is widely believed to play a central role in the pathogenesis of cardiovascular diseases including myocardial infarction and atherosclerosis. In the inflamed microenvironments, cells may undergo phenotypic transitions in the context of diseases. In the infarcted hearts, macrophage-myofibroblast transition (MMT) was identified by single cell-RNA sequencing. MMT is the important event to cardiac repair, while aberrant MMT is associated with pathologic fibrosis. In atherosclerosis, the stability of fibrous cap is a critical determinant of acute vascular events. The phenotypic changes of vascular smooth muscle cells (VSMCs) toward macrophage-like cells in the fibrous cap is closely related with the rupture of vulnerable plaque.
Based on the understanding of cell transition, several approaches to normalize or modulate the cell transition showed the remarkable therapeutic interventions.
This talk will introduce the current state of knowledge of the cellular transitions, with a primary focus on the cell heterogeneity to provide a foundation for a deeper understanding of the enigmatic cardiovascular diseases.

Keywords: inflammation, cardiovascular, cell transition

S13-4

Cell therapeutics for myocardial infarction based on hPSC-derived multicellular spheroids

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Ischemic heart disease remains the primary cause of morbidity and mortality worldwide. Despite significant advancements in phar-

macological and revascularization techniques in the late 20th century, heart failure (HF) prevalence after myocardial infarction (MI) has gradually increased over the last two decades. After ischemic injury, pathological remodeling results in cardiomyocytes (CMs) loss and fibrosis, which leads to impaired heart function. Unfortunately, there are no clinical therapies to regenerate CMs to date, and the adult heart's limited turnover rate of CMs hinders its ability to self-regenerate. Spheroids are 3D structures that can be generated from stem cells or tissue-derived cells in vitro, and they can recapitulate some of the structural and functional characteristics of the corresponding organs. In the context of cardiac regeneration, spheroids have been proposed as a potential approach for generating functional heart tissue for transplantation. Cardiac spheroids can be generated by differentiating human pluripotent stem cells (hPSCs) into various cardiac cell types including CMs, endothelial cells (ECs) and cardiac fibroblasts (CFs), and assembling them into 3D structures that resemble the architecture of the heart. Increasing interest has been directed towards non-CM cell types in driving myocardial renewal. We will discuss the therapeutic potential of 3D cardiac spheroids derived from hPSCs for cardiac regeneration and the limitations to establish their safety and efficacy in preclinical and clinical settings including optimization, maturation, and integration with the host tissue.

Keywords: ischemic heart disease, microenvironment, cardiomyocytes, cardiac spheroids, cardiac regeneration

S13-5

KAI1 (CD82) is a key molecule to switch angiogenic milieu to quiescent state

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Little is known about endogenous inhibitors of angiogenic growth factors. In this study, we identified a novel endogenous anti-angiogenic factor, KAI1 expressed in pericytes and clarified its underlying mechanism and clinical significance. There are many pro-angiogenic factors in the cancer microenvironment. In these environments, the gene expression of KAI1 is reduced by methylation of the KAI1 promoter. The protein level of KAI1 is rapidly reduced by endocytosis and degradation.
A peptide derived from the large extracellular loop of KAI1 has been shown to have anti-angiogenic effects to block the progression of breast cancer and retinal neovascularization in vivo. Therefore, KAI1 from PC is a novel molecular regulator that counterbalances the effect of angiogenic factors.

Keywords: KAI1, peptide, perycyte, angiogenesis, retinopathy

S14-1

γ-TuRC regulates radial migration and neuronal maturation during mammalian cortical development

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The γ-tubulin ring complex (γ-TuRC) is a multi-subunit protein complex composed of γ-tubulin and γ-tubulin complex proteins (GCPs, GCP2-6). γ-TuRC promotes microtubule assembly by serving as a template that allows efficient nucleation and elongation of α/β-tubulins into microtubule filaments. Thus, γ-TuRC plays a crucial role in various cellular processes such as division, polarization, migration and differentiation. Mutations in γ-TuRC core subunits and its activator cause brain developmental disorders known as malformations of cortical development. However, it remains unclear how γ-TuRC is involved in different stages of cortex formation. Here, we investigate the function of γ-TuRC in key events of cortical development during mouse embryogenesis, including progenitor proliferation, multipolar-to-bipolar transition, radial migration and neuronal maturation. Knockdown of individual γ-TuRC subunits severely delays neuronal migration in the developing brain. Using live imaging of brain slices, we found that GCP2-depleted cells remain motile but are unable to orient and migrate radially. Interestingly, these cells also fail to differentiate into the neuronal lineage. Furthermore, analysis of human GCP2 variants from microcephaly patients revealed that the disease mutations exhibit migration defects similar to GCP2 loss of function. Using in silico molecular dynamics simulation of the cryo-EM structure, *in vitro* NanoBiT-based γ-TuRC assembly assay, and microtubule nucleation assay in cells, we demonstrated that the *in vivo* radial migration defects are attributed to impaired γ-TuRC assembly and γ-TuRC-dependent microtubule nucleation. Collectively, our findings indicate that γ-TuRC is indispensable for radial migration and neuronal maturation during cortical development, thus providing novel molecular insights into how γ-TuRC controls brain development.

Keywords: γ-TuRC, microtubule, cortical development, radial migration, neuronal differentiation

S14-2

Supramolecular biomaterials for injured brain regeneration

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Ischemic stroke leads to acute neuron death and forms an injured core, triggering delayed cell death at the penumbra. The limited regenerative properties of the brain hinder the recovery of impaired brain functions after ischemic stroke. However, recent rodent intervention studies have shown that manipulating the extracellular environment during the subacute phase can enhance the regenerative potential of the injured brain. In this symposium, I present a rational design for artificial extracellular matrix (ECM) mimics using supramolecular peptidic scaffolds that self-assemble through non-covalent bonds to form hydrogels. We have developed a cell-adhesive fiber-forming peptide that mimics the jigsaw-shaped hydrophobic surface in the dovetail-packing motif of glycophorin A as an artificial ECM for regenerative therapy. The jigsaw-shaped self-assembling peptide forms several-micrometer-long supramolecular nanofibers through a helix-to-strand transition to form a hydrogel under physiological conditions that is three-dimensionally homogeneously dispersed. The molecular- and macro-scale supramolecular properties of the jigsaw-shaped self-assembling peptide hydrogel facilitate the efficient incorporation and sustained release of vascular endothelial growth factor, and have shown cell transplantation-free regenerative therapeutic effects on a subacute-chronic phase mouse stroke model. Supramolecular peptide hydrogels that mimic ECMs and promote angiogenesis may represent a new drug modality for regenerative medicine in various tissues.

Keywords: ischemic stroke, regeneration, biomaterials, self-assembling peptide, angiogenesis

S14-3

Exploratory internal state encoded by hypothalamic SF-1 expressing neurons drives social investigative behaviors in mice

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The Steroidogenic factor 1 expressing neurons located in the dorsomedial/central parts of the ventromedial hypothalamus (VMH-SF1 neuron) are essential for maintaining energy homeostasis and driving innate behaviors. Previous studies have suggested that VMH-SF1 neurons encode a predator-orientated defensive state. However, it remains unknown whether these neurons respond to other hostile conditions and their behavioral relevance. In this study, we used in vivo calcium imaging with fiber photometry and a head-mounted miniature microscope to monitor the activities of the VMH-SF1 neurons in freely-roaming mice in response to predatory- or conspecific cues. We found that the VMH-SF1 neurons were activated by conspecific exposure, but induced only moderate responses when encountering predatory cues. Additionally, VMH-SF1 neuronal activities showed a strong temporal correlation with exploratory but not defensive behaviors. Conspecific and predatory cues recruited distinct subsets of VMH-SF1 neurons, and these subpopulations reliably encoded the identity of the stimulus. However, manipulating all VMH-SF1 neurons altered animals' defensive states, implying that conventional manipulations without selectivity to stimulus-selective subsets might not truly reflect the behaviors controlled by these neurons in vivo. We suggest that the VMH-SF1 neuronal population is heterogeneous and can be divided into several functionally distinct subsets based on their stimulus selectivity. One subset prompts animals' investigative behaviors upon detecting the presence of their preferred external stimulus, while another facilitates defensive state-related behaviors.

Keywords: innate behavior, SF1, hypothalamus, social behavior

S14-4

Activation of K_{ATP} channel in brown adipose tissue attenuates depression-like symptoms through dopaminergic neurons in the ventral tegmental area

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Epidemiological evidence suggests that comorbidity of obesity and depression is extremely common and continues to grow in prevalence. However, the mechanisms connecting these two conditions are unknown. Our previous publication has shown that glibenclamide (GB) infusion in the interscapular brown adipose tissue (BAT) attenuates high-fat diet (HFD)-induced depression-like behaviors. In the follow-up study, we sought to explore the underlying mechanism. After mice were fed with either chow or HFD for 12 weeks, the miniosmotic pump filled with BODIPY-GB was implanted in the interscapular BAT. BODIPY-GB was co-expressed with dopaminergic (DA) neurons in the ventral tegmental area (VTA). We found the metabolic regulator, FGF21, was increased in the HFD+GB group. HFD-fed mice were implanted with mini-osmotic pumps containing His-tagged FGF21 recombinant protein in the interscapular BAT for two weeks, followed by intraperitoneal administration of FGF21 (3 mg/kg) for four consecutive days. This treatment regimen reduced signs of metabolic dysfunction, attenuated depressive-like behaviors, and restored mesolimbic dopaminergic projections in HFD-fed mice. FGF21 treatments also rescued dysregulation of FGF21 receptors, FGFR1, and the co-receptor β-klotho, in the VTA. In summary, forced closure of K_{ATP} channels by GB treatment led to increases in FGF21 mRNA levels, protein levels and release from BAT, followed by correction of FGF21 receptor dimers in the VTA DA neurons to attenuate depression-like behaviors.

Keywords: K_{ATP} channels, depression, FGF21, high fat diet, dopamine

S15-1

Intracellular thermometry with fluorescent molecular thermometers: From basics to biological applications

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Intracellular temperature has received widespread attention because it is assumed to be related to many cellular activities and the health status of cells [1]. Fluorescent molecular thermometers [2,3] are promising analytical tools for intracellular thermometry because of high-temperature resolution (better than 1°C) and high spatial resolution (molecular scale in principle). In the last 15 years, we first developed a fluorescent polymeric thermometer by combining a thermoresponsive polymer with an environment-sensitive fluorophore and performed intracellular mapping of mammalian cells using fluorescence lifetime imaging microscopy [4]. Then, a cationic fluorescent polymeric thermometer that could enter living cells was also prepared [5]. Finally, a ratiometric fluorescent polymeric thermometer that enabled intracellular thermometry with a standard fluorescence microscope or a laser confocal microscope was developed [6]. These fluorescent thermometers are now commercially available [7] and have been applied to biological studies, including thermal signaling [8]. Our laboratory contributed to thermal biology by utilizing intracellular thermometry with the fluorescent thermometers developed: Mechanisms of thermogenesis in brown adipocytes [9,10], temperature-dependent neuronal differentiation, and metabolism-dependent intracellular thermogenesis. A new class of an uncoupler, BAM-15 ((2-fluorophenyl){6-[(2-fluorophenyl)amino](1,2,5-oxadiazolo[3,4-e]pyrazin-5-yl)}¬amine), was also developed. Compared to conventional uncouplers, BAM-15 is useful for studying the mechanism of mitochondrial thermogenesis as it specifically works toward the mitochondrial membrane but does not depolarize the plasma membrane [11].

Keywords: fluorescence, temperature, thermometry, cell, fluorescent probe

S15-2

N-Terminal formyl-methionine acts as a critical determinant for the ribosome-associated protein quality control

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In bacteria, nascent proteins have N-terminal (Nt) formyl-methionine (fMet) that is pre-made before translation. The Nt-fMet of bacterial proteins acts as a specific degradation signal, termed fMet/N-degron. By

contrast, proteins synthesized by cytosolic ribosomes in eukaryotes are generally thought to have non-formylated Nt-Met. We previously found that the budding yeast *Saccharomyces cerevisiae* formyltransferase Fmt1 can also produce Nt-formylated proteins in the cytosol, even though it is normally imported into mitochondria. Cytosolic N-terminal formylation of nascent polypeptides adapts yeast cells to cold stress. However, the regulatory mechanism for how cold stress regulates fMet-containing protein synthesis is unknown. Here, we show that low temperature allows cytosolic ribosomes to initiate protein synthesis with fMet. As a result, when a fMet-containing polypeptide emerges from the ribosomal exit tunnel, its Nt-fMet stalls and splits ribosome, promoting stress granule formation. Accumulating stress granules make yeast cells more resistant to cold stress, revealing a novel mechanism by which yeast cells adapt to cold stress.

Keywords: N-formylmethionine, fMet/N-degron, ribosome-associated quality control, protein synthesis, protein degradation

S15-3

Super-resolved mitochondrial proteome mapping in mice muscle tissues

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Local proteome mapping using proximity is now a viable strategy for profiling subcellular proteomes with high resolution [1]. We generated transgenic mice (MAX-Tg) expressing a mitochondrial matrix-targeted ascorbate peroxidase (MTS-APEX2) to analyze tissue-specific matrix proteomes. Desthiobiotin-phenol labeling of muscle tissues from MAX-Tg mice allowed for the efficient profiling of tissue-specific matrix proteome. Comparative analysis of matrix proteomes from MAX-Tg muscle tissues revealed differential enrichment of mitochondrial proteins related to energy production. We identified that Reticulon 4 interacting protein 1 (RTN4IP1), also known as Optic Atrophy-10 (OPA10), is highly enriched in the mitochondrial matrix of muscle tissues and is an NADPH oxidoreductase. Interactome analysis and in vitro enzymatic assays revealed an essential role for RTN4IP1 in coenzyme Q (CoQ) biosynthesis by regulating the O-methylation activity of COQ3. Rtn4ip1 knockout C2C12 myoblasts had markedly decreased CoQ9 levels and impaired cellular respiration, which was rescued by exogenous CoQ treatment. Muscle-specific knockdown of the drosophila Rtn4ip1 ortholog resulted in impaired muscle function which was reversed by dietary supplementation with soluble CoQ. Collectively, RTN4IP1 is a mitochondrial antioxidant NAD(P)H oxidoreductase that is essential for supporting mitochondrial respiration activity in muscle tissue [2].

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Keywords: mitochondria, spatial proteomics, tissue-specific, proximity labeling, coenzyme Q

S15-4

Regulation of brown adipose tissue thermogenesis by mitochondrial calcium uniporter

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The role of the mitochondrial calcium uniporter (MCU) in facilitating calcium entry into the mitochondrial matrix is well understood, but its connection to heat production in thermogenic adipose tissues has remained a complex and underexplored area. Our study sheds light on how MCU orchestrates mitochondrial functions in brown adipocytes through the regulation of mitochondrial reactive oxygen species (mtROS). By employing brown adipose tissue-specific Mcu knockout (*Mcu* BKO) mice, we uncovered a decrease in oxygen consumption and heat production, aligned with the downregulated expression of genes vital to beta-oxidation and thermogenesis. Furthermore, the *Mcu* BKO model revealed a distinct cold-intolerance phenotype, associated with reduced mtROS levels. We identified that the expression of thermogenic genes is critically regulated by mtROS. Taken together, our findings present a compelling insight into the role of MCU in modulating mtROS formation, a pathway that mediates the cellular response through mitonuclear signaling and mitochondrial activation.

Keywords: mitochondria, MCU, brown adipose tissue, mtROS, thermogenesis

S16-1

The role of exercise and nutraceuticals in modulating physiological ROS levels

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Reactive oxygen species (ROS) are oxygen derivatives that arise intrinsically from the oxidative phosphorylation process and extrinsically as a response to xenobiotics and pollution. ROS is involved in various conditions such as exercise, aging, inflammation, and neurodegenerative diseases. Meanwhile, physical activity, specifically exercise, can modulate ROS. The impact of exercise on ROS varies from harmful to beneficial and depends on the type of exercise as they induce different types of ROS. Long-term exercise regulates signaling pathways that enhance antioxidant defense systems and control ROS production. Thus, understanding of physiological or pathological ROS roles are important. In addition, nutraceuticals supplement are believed to have a significant role in regulating this ROS connecting with Aging or other risk factor. Our discussion will focus on how exercise can regulate ROS and which type of exercise has a role in delaying the aging process as well as exploring role of neutraceuticals. We expose the impact of nutraceutical antioxidant agents that likely enhance the benefit of exercise. The nutraceutical antioxidants agents that likely enhance the benefit of exercise are creatine, whey, and ascorbic acid. Exercise is rewarding for the aging population concerning increasing their quality of life. Special consideration to exercise needs to be given to the type of exercise, and the exercise must be done continuously.

Keywords: nutraceuticals, exercise, ROS, Hormesis, autophagy

S16-2

Mitochondrial protein import and its physiological significance on mitochondrial and cell function

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Mitochondria is an essential organelle which plays a role in metabolism and supplies cells with energy in the form of adenosine triphosphate. Mitochondria repeats constantly fusion and fission. The control mechanism of its mitochondrial dynamics regulates not only mitochondrial quality control but also various important functions for cells such as apoptosis, respiratory activity, lipid metabolism, and iron metabolism. Furthermore, mitochondria have their own DNA (mitDNA). However, since the proteins encoded by mitDNA are very limited, most of the proteins that consist of mitochondria must be supplied from nuclear DNA. As the elucidation of the protein transport mechanism from nuclear DNA progresses, the structure of the transport complex involved and the mechanism for sorting the transported proteins are being clarified. Mitochondria cleave off the compromised sites by fission and are eliminated (mitophagy). Mitophagy is also induced when unfolding proteins are transported and accumulated in mitochondria, and these mechanisms have been shown to suppress intracellular accumulation of defective proteins in such as myocardium and neuron. Recently, our research team confirmed that a mitochondrial non-constituent protein (Mb) has been present in the mitochondria. Mb is a monomeric hemoprotein, which is expressed rich in oxidative skeletal muscle, cardiac and brown adipose tissues. Mb is responsible for oxygen storage, buffering intracellular oxygen concentrations and facilitating oxygen diffusion. Mitochondrial Mb interacts with subunits of Complex IV and upregulates mitochondrial respiratory activity. Endurance training further increases endogenous Mb in mitochondria and increases respiratory activity. Surprisingly, the accumulation of Mb, which is not a mitochondrial protein, enhances mitochondrial function without inducing mitophagy. It has been reported that proteins such as Mb exist in mitochondria that are not originally transported to mitochondria. The transport mechanisms of these non-constitutive proteins and their physiological functions within the mitochondria have not been fully elucidated. This symposium, by presenting some of evidence relating mitophagy-induced by protein transport or to preserve proteins to modify mitochondrial functions, will be make an opportunity to reconsider significance of protein transport into mitochondria.

Keywords: complex IV, exercise, myoglobin, protein transport, TOM

S16-3

Autophagic protein ULK1: A promising target to regulate cellular senescence

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Autophagy is a recycling process that maintains cellular homeostasis by regulating the turnover of long-lived proteins and damaged or dysfunctional cellular components. During times of nutrient shortage, autophagy promotes cell health and survival by providing constituents required to maintain metabolism, and the down-regulation of autophagy is associated with several age-related metabolic diseases. Autophagy is involved in response to starvation, cell growth, and anti-aging mechanisms. Autophagy and telomere maintenance are two cellular survival processes that show a strong correlation between human aging and cancer growth, however, their causal relationship remains unclear. To gain insight into the relationship between autophagy and the processes regulating cellular proliferation, we utilized an unbiased transcriptomics approach to study differential genes and pathways affected by autophagy inhibition in hepatic cells. Here, we discovered a novel role of autophagy genes in regulating telomere extension and maintenance pathways. The pharmacological inhibition of ULK1 (Unc-51 like autophagy activating kinase 1) attenuated human telomerase reverse transcriptase (hTERT) gene expression and telomerase activity in HepG2 cells. The suppression of telomerase activity upon ULK1 inhibition was also associated with telomere shortening and the onset of cellular senescence in HepG2 cells. These results establish a direct link between autophagy and cellular longevity via the regulation of cellular telomerase activity in mammalian cells. The modulation of telomerase expression in normal cells may extend the lifespan in patients with inherited telomere spectrum disorders, cancers, and aid in the treatment of age-related metabolic diseases.

Keywords: autophagy, Ulk1, telomere, senescence, hepatic cells

S16-4

Physiologic aspect of nutraceutical agents as cell energetic, mitochondria for exercise performance

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In response to physiological stressors, skeletal muscle has the potential to elicit a wide variety of adaptive responses, such as the biogenesis of mitochondria or clearance of damaged mitochondria to promote healthy muscle. In addition, muscle turnover can prevent metabolic imbalance, cardiovascular disease, and accelerated aging. Skeletal muscle is a highly malleable tissue, capable of considerable metabolic and morphological adaptations in response to repeated bouts of contractile activity (for example, exercise). It is well-established that chronic contractile activity, in the form of repeated bouts of endurance exercise, usually interspersed with recovery periods, results in the altered expression of a wide variety of gene products, leading to an altered muscle phenotype with improved resistance to fatigue. This improved endurance is highly correlated with increased muscle mitochondrial density and enzyme activity, referred to as 'mitochondrial biogenesis'. Exercise stimulates mitochondrial biogenesis and increases mitochondrial respiratory function and content. Several nutrients, such as polyphenols, also have an ability to induce mitochondrial biogenesis in skeletal muscle. Our research interested to investigated nutraceutical come from our original natural Indonesia included curcumin, moringa, nutmeg etc. The polyphenol curcumin is a natural antioxidant that exhibits various pharmacological activities and therapeutic properties. Our previous study has shown that curcumin increases mitochondrial content in rat gastrocnemius muscle and curcumin combined with endurance training additively enhances the effect of exercise on mitochondrial biogenesis. Our previous study concludes that curcumin treatment induces mitochondrial biogenesis through increasing cAMP levels by an inhibition of PDE4A and subsequent activation of PKA/LKB-1/AMPK pathway in skeletal muscle. We also found that curcumin and endurance exercise act as either an inhibitor or an enhancer of PDE4A, respectively, although both treatments stimulate cAMP production. On the other hand, our other previous study focuses to investigate the effect of moringa leaf extract and moderate intensity exercise on histopathological appearance and autophagy gene expression of

wistar rat liver. This study presented that moderate intensity exercise induced changes on histopathological appearance of wistar rat liver that might be associated with physiological inflammation. Moringa, with its antioxidant properties, combined with increased autophagy might improve histopathological changes in moringa + exercise group. Furthermore, moringa also potentially to increase total oxphos unit expression with correlation to increasing mitochondria biogenesis. Based on several our previous study we suggested that nutraceutical agent included curcumin, moringa and nutmeg potentially to improve mitochondrial biogenesis to increase exercise performance.

Keywords: mitochondria biogenesis, curcumin, moringga, exercise

S17-1

Immunotherpay drives metabolic reprogramming of therapy-refractory tumor cells

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Tumor cells undergo molecular evolution under various therapeutic pressures such as immune-editing caused by intrinsic or extrinsic anti-tumor immunity. Due to the exquisite specificity and potency of the immune system, cancer immunotherapy is in theory the most precise and powerful approach for controlling cancer metastasis and minimal residual disease, as well as for preventing relapse. However, current data from clinical trials indicate that immunotherapy rarely yields significant benefits for cancer patients in terms of tumor progression and long-term survival. The existing paradigm is that the poor clinical outcomes of immunotherapy are primarily caused by mechanisms of peripheral immune tolerance, especially within tumor microenvironment (TME). Therefore, the identification of clinically available targets that restrict antitumor immunity is required to develop potential combination therapies. Here, in this presentation, using transcriptomic data on patients with cancer treated with programmed cell death protein 1 (PD-1) therapy and newly established mouse preclinical anti-PD-1 therapy-refractory models, we identified NANOG as a factor restricting the amplification of the antitumor immunity cycle, thereby contributing to the immune-refractory feature of the TME. Mechanistically, NANOG induced insufficient T cell infiltration and resistance to CTL-mediated killing via the histone deacetylase 1-dependent (HDAC1-dependent) regulation of CXCL10 and MCL1, respectively. Moreover, NANOG reprogramed the metabolism of the refractory tumor cells by inducing the epigenetic loss of the ATP synthase subunit ATP5H, which leads to ROS accumulation and HIF-1α stabilization under normoxia. Importantly, HDAC1 inhibition using an actionable agent sensitized NANOG+ immune-refractory tumors to PD-1 blockade by reversing the abnormal metabolism and reinvigorating the antitumor immunity cycle. Thus, strategies that reverse TME including metabolism of tumor cells could improve the clinical management of immunotherapy-refractory cancer.

Keywords: immune checkpoint blockade, immunotherapy-refractory cancer, ATP synthase, NANOG, HIF

S17-2

Targeting the gut microbiota on cellular heterogeneity in cancer

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The mammalian gastrointestinal tract, the site of digestion and nutrient absorption, is home to a microbial ecosystem that enhances resistance to infection, inflammation, allergy, cancer, and metabolic diseases. Commensal bacteria are key participants in the digestion of food, and are responsible for the extraction and synthesis of nutrients and other metabolites that are essential for the maintenance of mammalian health. Over the past decade, the connection between various disorders and gut microbiota has become a major focus of biomedical research. Because of the complexity of the microbiota community, however, the underlying molecular mechanisms by which the gut microbiota is associated with diseases remain poorly understood. In this talk, I summarize recent studies that investigate the role of the microbiota in animal models of disease and discuss relevant therapeutic targets for future research.

Keywords: mucosal immunity, gut microbiota, cancer, high-fat diet, metabolites

S17-3

Microbiota and gastrointestinal cancer: pathology, prognosis, and treatment

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Powered by high-speed high-throughput next-generation genomic technologies, life science and biotechnology are being transformed. In our laboratory, we apply genomic and metagenomic tools to study model microbes and microbial communities. The microbiome, comprised of the microbiota, their collective genomes called the metagenome, and the macromolecules and metabolites they produce, is an integral part of our body and the ecosystem. Microorganisms have shaped our planet's biosphere and mediate biogeochemical cycling and energy flow in nature. Systems understanding of host physiology can be possible only if the microbial counterparts that reside in are fully appreciated and both are considered as a unit, i.e. holobiont. Recent analyses of metagenome-assembled genomes and meta-omics data, as well as more traditional community data, revealed that a myriad of microbial members, mutualistic, commensal, or pathogenic, play pivotal roles in host health, disease, and aging. Host-microbiota relationships in the human gastrointestinal tract, as well as the dynamics of microbial communities, will be presented as examples. In the talk, efforts to develop probiotics or more preferably pharmabiotics for the prevention or treatment of various diseases including gastrointestinal cancers and aging will also be presented. Synthetic biology concepts and toolkits enable us to modulate the microbiome to maintain or regain homeostasis (eubiosis and rebiosis, respectively), and even to transform it to become preventive or curative.

S24-1

Innovative approaches in physiology teaching – the supporting role of technology

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For most physiology academics, teaching physiology has increasingly become an important part of our career, that is, in addition to other research responsibilities. A huge portion of our time is now dedicated to engaging students in diverse settings of learning and teaching. In just a few years, we have seen such a rapid pedagogical evolution unlike what the world has seen the whole of past few decades. Progressing from an instructor-centred delivery system, we have now transitioned to using various forms of innovation including online delivery, remote teaching, flipped classrooms and other different instructional practices. While integration of technology into physiology learning and teaching is not new, the increasing interdependence on these teaching tools is now unprecedented. With the aid of open-source simulations, 3D printed models, virtual reality, and other available experimental setups – teaching physiology has become increasingly interactive and student-centred. The use of web-based virtual learning environment and learning management systems further enhance student experience. As we embrace the use of technology to empower students through collaborative and innovative learning approaches, our aim remains – that is to facilitate learning in understanding physiological concepts and to train practical skills needed to develop physiology related competencies.

Keywords: innovation, technology, simulations, 3D printing

S24-2

Application of AI technology on lab teaching in Physiology

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Human motion is an essential element in Physiology. However, the limitation of analytic tools cannot provide medical students a comprehensive viewpoint on how the motion impacts respiratory and cardiovascular systems. With rapid development of deep learning in artificial technology (AI), human skeleton information in real-time camera images is constructed into skeleton-joint models. The 18-joint model coupled with mathematical algorithms becomes a clear gesture recognition system which has been extensively applied in physical rehabilitation and imitation games. My AI team further added a counting tool to the gesture recognition system and transformed the system to a measurable device which can evaluate the performance of a specific motion. The in-house developed AI-based evaluation tool with AR goggle was used in Physiology Lab to explore how the exercise intensity of a specific form of exercise on respiration rate, oxygen saturation, blood pressure, and heart rate. This is the first time ever for students to compare the different exercises on various physiological system. Although all participants were aware of future needs of artificial intelligence on health care, the learning experiences helps them realize how the AI technology can be applied in healthcare.

Keywords: AI technology, physiology education, laboratory teaching, exercise, skeleton model

S24-3

Modules of physiology laboratory using zebrafish

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Osaka Medical and Pharmaceutical University, Japan

While laboratory modules are indispensable for effective education of physiology at medical schools, various factors including cost and animal welfare render their implementation challenging. I will introduce lab modules we have employed at Osaka Medical and Pharmaceutical University.
4 modules out of 6 at OMPU are performed on humans, recruiting students as volunteers. Students perform half-day experiments on electromyogram, urine analysis, electrocardiogram and motor learning. In 2 modules, we employ zebrafish, a small sized tropical fish, as experimental model which we use also for research purpose and maintain in the self-circulating maintenance system. In one module, we use zebrafish embryos (~ 2 mm body length) and students observe autonomous rhythmicity of central pattern generator by dissecting the head region. Students record the swimming pattern of headless body trunks and compare with normal intact siblings. In another module, students isolate testes from adult male zebrafish, and examine the sensitivity of sperm motility to high temperature.

Keywords: zebrafish

S24-4

Ensuring cognitive presence in class - judicious use of technology

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Cognitive presence is a four-stage process of practical inquiry that measures how well students can generate and reinforce meaning through discussion and reflection. Triggering event, investigation, integration, and resolution make up cognitive presence. It has gotten much harder for teachers to assure cognitive presence and student participation in classroom instruction as enrolment in undergraduate and graduate programmes has increased and educational objectives have changed to include student-centred teaching and learning activities.
The usual practice that teachers adopt is promoting conversations on the lessons being taught and allowing students in exploring and comprehending the relationships between ideas. They ask pupils to defend and articulate their ideas by posting random questions, enhancing knowledge and memory in students through visualisation. However, in an hour-long class neither of these techniques offer a satisfactory involvement of students when batch strength is very high. It is during this time that cognitive presence becomes important as compared to teaching presence and social presence in ensuring engaging and effective learning.
Technology helps in ensuring this building up of a community of inquiry (CoI) through multiples methods like Slido, Kahoot, Vevox, Miro and Padlet. These apps help in quizzing, polling and quick attention buzzes interspersing the lecture of one hour to be distributed into three or four sections of concept-based teaching. Teachers can ensure existing knowledge in students regarding a topic through a quiz set in the beginning of the class and build up the lesson accordingly. The other technique where technology helps is in the implementation of a case based flipped classroom or team-based learning approach.

The talk will discuss these methods in details with examples from the speaker's own experience in both onsite and online classes.

Keywords: cognitive, online, technology-enabled

S24-5

Integrated systems physiology - Sri Lankan experience

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Novice undergraduates in health sciences might find it difficult to integrate systems physiology with other basic disciplines and their application at the clinical level. If horizontal and vertical integration is ensured systematically in an undergraduate curriculum, it could guide students from the inception. The University of Colombo Faculty of Medicine (UCFM) revise the medical curriculum periodically. During the last revision in recent years, a system-based approach was introduced with core clinical cases while facilitating integration between anatomy and biochemistry and with other streams to achieve the mission of UCFM.

The COVID-19 pandemic provided an opportunity to adopt new technology; the ZOOM platform to facilitate teaching/learning activities including formative assessments. Video-based sessions were also created and used on the LMS platform to facilitate practical concepts until physical sessions were possible. Student seminars based on clinical scenarios were introduced for students to appreciate the application of knowledge in actual clinical setups.

Exposure of students to advanced technology in practical sessions facilitates the understanding of systems physiology by witnessing them being utilized in real-life patient investigations. Examples include the use of autonomic function testing by power lab to discuss cardiovascular regulation, cardiopulmonary exercise testing to teach exercise physiology, nerve conduction studies, and electroencephalogram in neurophysiology and imaging in gastrointestinal and renal physiology. These create interest among students and facilitate learning of applied system physiology. Physiologists specializing in different disciplines promote the use and application of advanced technology in integrating systems physiology.

Keywords: teaching and learning, systems physiology, technology, integration, applied physiology

S24-6

Optimal blended subject design (non-face-to-face real-time class, recorded video, and face-to-face assessment combination model) and effect analysis on mastery learning

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Background: According to ' Bloom 's Mastery Learning Model', students should reach the stage of mastering the information of that level (the stage of mastering 90% of the knowledge evaluation) before moving on to the next learning. Even in medical schools with a large amount of academic work, it is a challenging task if an appropriate level of learning tasks and assessments, efficient non-face-to-face video classes and real-time student participation classes are designed in detail, so researchers are aiming for complete learning to study physiology in a hybrid form. designed and operated.

Methods: In 2020 and 2021, the authors conducted 'Human Functions', 'Basic Circulatory Respiration', and 'Structure and Function of the Nervous System' courses at CHA Medical School in a branded format (non-face-to-face recorded videos, real-time classes, and face-to-face evaluations) was designed and operated. Based on Carroll's school learning model and Gagne's 9 events, the class was designed and conducted as a video class, and a survey was conducted after the course was completed to find out about the learning effect (42/44 respondents, 95%, 5 point scale), the results and academic achievement were analyzed.

Results: The subject consists of diagnostic evaluation, instruction, summative evaluation, feedback, and remediation. Classes and summative assessments were designed in consideration of learning ability and speed. One set consists of (1) video lessons, (2) student active participation classes using small group online spaces to utilize knowledge and (3) face-to-face evaluation. The video class is preparation for learning, class, formative evaluation and feedback, and end of class. As a result of the survey, most respondents (4.76) said that the composition of the curriculum was very suitable for learning. They responded that the designed class was more helpful than the general class (4.79), and that the video class had no schedule restrictions (4.98) and could be reviewed (4.93), so it was effective for learning (4.83). It was an appropriate teaching method. The average score of the summative evaluation was 90.0, and 63.6% of the students scored 90 or higher. In terms of academic achievement, 77.2% of students scored 90 points or higher, which was about 13% less than the target of 90% of complete learning.

Conclusions: In order to consider students' academic ability and speed and to increase practical academic efficiency, the class was divided into 4 stages and designed in detail into about 10 components. The curriculum consisted of video classes, online small group activities, and face-to-face evaluations. Most of the students gave positive evaluations, and their academic achievement improved close to perfect learning. Through more meticulous design of classes and subjects, faithful operation, and active participation and feedback from students, it is expected that medical schools will be able to achieve results close to perfect learning.

Keywords: blended learning, mastery learning, class design

S56-1

Real-time intravital microscopy with suction-assisted imaging windows for thoracic organ imaging

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Intravital microscopy is a unique imaging technique to visualize various *in vivo* cellular-level dynamics such as cell trafficking, cell-to-cell or cell-to-microenvironment interactions in a live animal. Intravital imaging of cellular dynamics in a natural physiological microenvironment can provide unprecedented insights in the dynamic pathophysiology of human diseases those were impossible to obtain through conventional histological observation of *ex vivo* samples or in vitro culture system. During the last decade, the intravital microscopy has become a highly valuable, indispensable technique in wide areas of biomedical sciences such as immunology, neuroscience, developmental and tumor biology. Notably, *in vivo* visualizations of gene expression, protein activity, cell trafficking, cellular interactions and various physiological responses

to external stimuli have been successfully achieved. Additionally, it is a unique tool for the development of new therapeutics and diagnostics by providing improved accuracy and reliability in *in vivo* target validation with delivery monitoring and efficacy assessment. It has been used to directly analyze the delivery and efficacy of new biopharmaceuticals such as antibodies, cell therapy, gene therapy, nucleic acids and exosome in an *in vivo* microenvironment.
In this talk, a custom-built real-time intravital two-photon and confocal microscopy system will be introduced. The imaging system has been extensively optimized for *in vivo* cellular-level imaging of internal organs in the live animal model for various human diseases. It can acquire real-time video-rate multi-color sub-micron resolution microscopic images in a live animal model with automatic motion compensation, enabling direct imaging analysis of complex cancer immune-microenvironment consisted of various immune cells, stromal cells, vascular cells and extracellular matrix. Intravital imaging of various organs including skin, liver, spleen, pancreas, kidney, small intestine, colon, retina, lymph node, brain, and bone marrow will be briefly introduced. Notably, the video-rate imaging capability of the developed intravital microscopy system with a suction-assisted imaging window enabled *in vivo* cellular-level imaging of thoracic organs, a beating heart and a breathing lung, in a live mouse. Pulmonary microcirculation and endothelial surface layer in pulmonary capillaries in normal and pathological condition were successfully visualized. It can provide novel insights to understand dynamic cellular-level pathophysiology of various human diseases and develop novel therapeutics to treat them.

Keywords: intravital microscopy, two-photon microscopy, in vivo imaging, circulation, cellular imaging

S56-2

Label-free 3D live cell imaging and quantification using holotomography

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Holotomography has emerged as a useful tool for imaging live specimens without additional pre-treatment, such as fixation, fluorescence labeling, and excitation. Holotomography can achieve label-free, high-resolution imaging of live specimens, including small organoids and fresh tissue samples in 3D. These samples can be observed for weeks without cellular damage caused by photoactivation. The high resolution (under 150 nm lateral) achieved through synthetic numerical aperture provides sufficient spatial information to distinguish various subcellular compartments such as nuclei, nucleoli, mitochondria, lipid droplets, etc. Furthermore, analysis of the measured individual cell data can elucidate the temporal 3D volumetric dynamics with the dry mass information.
This talk will present the latest development of a low-coherence holotomography imaging system, HT-X1, and its numerous applications to different types of biological specimens, ranging from unicellular organisms to multicellular specimens. Several case studies will be introduced, such as real-time quantification of subcellular lipid droplets and liquid-liquid phase separation, bacteria classification, and cytotoxicity assay. We will also discuss how low-coherence holotomography can be a versatile tool for many research fields combining downstream molecular analysis, such as cell biology, immunology, microbiology, material science, and in vitro diagnosis.

Keywords: holotomography, live cell imaging, 3D biology, organelle dynamics, quantification

S19-1

Unlocking the potential of gut microbial enzymes for precision treatment of metabolic diseases

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Current methods for modulating the gut microbiota, such as fecal microbiota transplantation (FMT) and antibiotics, come with challenges: FMT carries infection risks and has a complex composition, while antibiotic misuse can lead to resistance and the emergence of superbugs. These approaches generally alter the microbial community indiscriminately. This raises the question: can we regulate specific microbial strains without affecting the overall community structure? Gut microbial enzymes are key players in nutrient metabolism and the generation of active metabolites, but their nuanced roles in host metabolism, including their potential for coevolutionary mimicry of host enzymes, remain largely unexplored. In this context, we introduce the concept of 'Microbial-Host-Isozyme' to focus on gut microbial enzymes crucial for nutrient metabolism and active metabolite production. Our research uncovers a wide variety of these isozymes in the gut, capable of mimicking host enzyme functions and even cross-species regulation of metabolic diseases. For example, microbial DPP4 degrades active GLP-1, impacting glucose tolerance. Traditional DPP4 inhibitors like Sitagliptin are ineffective against microbial DPP4, unveiling a new variable in treatment outcomes. Through high-throughput screening, we identified Dau-d4, a potent microbial DPP4 inhibitor that ameliorates glucose imbalances in mice without altering the overall microbial community. Furthermore, our work shows that nicotine accumulation in the gut activates AMPK, affecting the stability of its novel substrate SMPD3 and inducing NASH. We also discovered *Bacteroides xylanisolvens*, a gut microbe with a nicotine-degrading enzyme, NicX, opening a new potential therapeutic avenue. Our work offers the prospect of targeted microbial strain regulation, providing new avenues for the precision treatment of metabolic diseases.

Keywords: microbial-host-isozyme, gut microbial enzymes, microbial dpp4

S19-2

Tumor – microbiome interactions in breast carcinoma

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Neoplastic diseases, as breast carcinoma, are characterized by changes to the microbiome in multiple microbial compartments that is termed oncobiosis. There are bidirectional interactions between the oncobiome and the tumor cells. The host's lifestyle choices (hygiene, nutrition, etc.), medication and the host's immune system and metabolism influence the composition and function of the microbiome. The microbiome modulate immune functions and antitumor immunity, furthermore, secretes metabolites that, through a similar mechanism as hormones, influence the behavior of tumors. The first mention involvement of oncobiosis in progression of breast cancer dates back to 1971 describing that the microbiome of breast cancer patients can deconjugate and reactive excreted estrogens. Our work assesses this complex relationship.
We have identified a set of bacterial metabolites that have cytostatic properties. These metabolites have diverse chemistry (amino acid

decarboxylation products, secondary bile acids, fermentation products of carbohydrates and fibers) and are active in low or submicromolar concentrations. The metabolites slow down cancer proliferation, but do not kill cancer cells, and their activity is specific for transformed cells, as the effects do not manifest on non-transformed, primary cells. The metabolites inhibit multiple cancer hallmarks, as epithelial-to-mesenchmyal transition, cancer cell metabolism, movement, diapedesis and metastasis formation. Each metabolite has an array of receptors. The signaling pathways of the metabolites involve mild oxidative stress due to the downregulation of NRF2 expression and activity. The elements of metabolite-elicited signaling and the oncobiome's biosynthetic decreases as a function of disease stage, grade and aggressivity.
Taken together, the oncobiome produces a set of cytostatic metabolites that have hormone-like properties; they are produced by a "gland", the oncobiome, and are then carried by the circulation to the site of action, the tumor cells. The secretory properties of the oncobiome decreases as a function of the state of the disease. Importantly, oncobiosis supports metastasis formation, hence, the management of oncobiosis can suppress the progression of the disease to stage IV.

Keywords: microbiome, breast cancer, dysbiosis, metastasis

S19-3

Microbiota-mediated immune modulation in mycobacterial infection

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The pulmonary infections caused by mycobacteria, including *Mycobacterium tuberculosis* (Mtb) and non-tuberculous mycobacteria (NTM), pose challenges in treatment due to their increased resistance to antibiotics. Recent evidence suggests that the gut-lung axis holds promise as a source of innovative host-directed therapeutics for combating various infectious diseases including mycobacterial infections. However, there is a scarcity of knowledge concerning the role of the gut-lung axis in host protective immunity, particularly in the context of identifying potential therapeutics against mycobacterial infections. In this study, we investigated the impact of gut microbes and their metabolites on conferring pulmonary immunity to mycobacterial infections. Metabolomics analysis revealed decreased levels of l-arginine in sera from patients with NTM pulmonary diseases (NTM-PD). To explore its potential therapeutic role, oral administration of l-arginine was administered into the mice with NTM-PD models, leading to significantly enhanced pulmonary antimicrobial activities. This enhancement was accompanied by the expansion of IFN-γ-producing effector T cells and a shift towards microbicidal (M1) macrophages in the lungs of an NTM-PD model in mice. Importantly, we observed that l-arginine administration enriched the gut microbiota composition with *Bifidobacterium* species. Strikingly, oral treatment with either *B. pseudolongum* or inosine demonstrated the ability to enhance antimicrobial pulmonary immune defense against NTM or Mtb. These findings highlight the crucial role of l-arginine-induced gut microbiota remodeling, particularly through the enrichment of *B. pseudolongum*, in bolstering pulmonary immune defense against mycobacterial infections. Our study sheds light on the promising potential of the gut-lung axis *in vivo*, offering new avenues for the development of host-directed therapeutics targeting mycobacterial infections.

Keywords: mycobacteria, metabolomics, microbiota, MTB

S19-4

Microbiota dysbiosis and gut barrier dysfunction: cause or consequence?

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A vast amount of bacteria, vira, archaea, and fungi inhabits the human gastrointestinal tract. The gut microbiota plays crucial role in human health by maintaining a mutually beneficial or symbiotic relationship with the host. Nevertheless, microbiota dysbiosis predispose to disease development, including inflammatory bowel diseases (IBDs) and colitis-associated cancers. The etiology of IBDs involves genetic, barrier, and microbiome interactions, resulting in chronic inflammatory flare in the gut. Patients with IBDs had a higher risk of developing colorectal carcinoma. Myosin light chain kinase (MLCK)-activated epithelial hyperpermeability, presence of intraepithelial bacteria and tight junctional damage, and microbiota dysbiosis were documented in patients and experimental models of IBD. However, the origins of microbiota dysbiosis and its relationship with epithelial barrier dysfunction remain elusive. Intestinal epithelia express two splice variants of long MLCK, of which the full-length MLCK1 mediated tight junctional opening whereas MLCK2 activation caused epithelial terminal web contraction, brush border fanning, and facilitated transepithelial bacterial endocytosis through cholesterol-rich lipid rafts and caveolin-dependent mechanisms. Our recent study has demonstrated that the MLCK-dependent bacterial endocytosis promoted invasive pathobiont conversion and shaped the colitogenic microbiota. Genotype-specific divergence of epithelial microbiota occurred before the emergence of distinct stool microbiota across littermates of MLCK-transgenic and normogenic mice. Moreover, circadian disruption was identified in response to invasive pathobionts for upregulation of proinflammatory cytokines and downregulation of anti-inflammatory glucocorticoid in epithelial cells. In conclusion, barrier dysfunction played a causative role in microbiota dysbiosis and colitis initiation.

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Keywords: microbiome, dysbiosis, barrier function, epithelial biology, inflammatory bowel diseases

S20-1

Role of lifestyle in neurogenesis and neuroplasticity

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Several studies have revealed that neuroplasticity is strongly influenced by the lifestyle and environment. Neuroplasticity can be hampered by aging and oxidative stress. The exposure to toxic, immobilization, and imbalances in nutrient also have a detrimental impact. However, neuroplasticity can be driven by various conditioned stressors. For example, physical activity and relative caloric restriction. Aerobic exercise combined with environmental enrichment may be more effective. Learning and training improve cognitive capacity because it spurs the elongation of dendrite spines to speed up connections. The structure of neurons is also built through the intake of nutrients and co-factors. The supply of these components depends on an adequate vascular system. A healthy diet such as caloric restriction with a balanced diet was more effective in growth and maintaining neuron structure. The brain still needs a balanced composition of nutrients for

energy metabolism and neurogenesis.

Keywords: neuroplasticity, lifestyle, exercise, caloric restrictions

S20-2

Moderate-intensity intermittent exercise induces neuroplasticity in rat model of hippocampal degeneration

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Background: Higher levels of presenilin-1 (PSEN-1) and phosphorylated tau (p-tau) as markers of Alzheimer's dementia (AD) in the hippocampus lead to the formation of amyloid plaques and neurofibrillary tangle which in turn induce neuroinflammation and cell death. In response to exercise, the release of signaling molecules known as exerkines is one of the key pathways of the neuroprotective effects of moderate-intensity intermittent exercise (MIIE). Several exerkines act as growth factors and anti-inflammatory cytokine that inhibit the development of dementia and reduce inflammation associated with increased dementia markers, thereby become an important pathway in the MIIE's preventive effect on hippocampus degeneration. MIIE supposed to help maintain cerebral microenvironment that promotes neuroplasticity by inhibiting elevated levels of AD markers thereby preventing AD pathogenesis.
Aim: This study aimed to investigate the neuroplasticity effects of MIIE on the spatial memory and protein level of AD markers in the hippocampus of trimethyltin (TMT)-induced rat model of dementia.
Methods: Male Sprague Dawley (SD) rats were randomly assigned into four groups, i.e. normal control (NC), exercise control (EC), TMT control (TC), and exercise & TMT (ET). Rats of the exercise groups (EC & ET) were forced to run on a treadmill for 30 minutes at maximum per day for 12 weeks. Intraperitoneal injection of 8 mg/kg BW TMT was administered as a single dose, 10 days before the last exercise treatment for TC and TE groups. The spatial memory of rats was examined using Morris Water Maze (MWM) test after the exercise period. After sacrifice, hippocampal tissue was dissected out and the level of hippocampal PSEN-1 and p-tau protein were measured using ELISA.
Results: TMT exposure induced spatial memory impairment as the TC group had the lowest result of MWM performance compared to other groups. Impaired spatial memory indicates hippocampus degeneration and represents as one of the AD manifestations. MIIE prevented the memory impairment effect of TMT exposure as the ET group had no significant different results in MWM performance compared to EC and NC group. The TE group had significantly lower levels of hippocampal AD markers, p-tau and PSEN-1 compared to the TC group ($p<0.05$).
Conclusion: MIIE prevents impaired spatial memory upon TMT exposure via preventing elevated levels of hippocampal AD markers. Prevention of elevated levels of hippocampal AD markers will maintain the microenvironment in the brain, hence supporting neuroplasticity.

Keywords: moderate-intensity intermittent exercise, trimethyltin-induced neurodegeneration, spatial memory, Alzheimer disease markers, neuroplasticity

S20-3

Anthocyanin modulation on behaviors and brain parameters in stress-induced animal model

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Stress has strong adverse effects on physical and mental health by triggering inflammation and oxidative stress. Plant bioactive compound well established as stress adaptogen to promotes stress recovery. Our studies focused on the role of total anthocyanin extract (ANC) from local cultivar of Purple Sweet Potato (PSP) in brain parameters and behavior of stress-induced stress animal. Stress was applied as two different points time. First point experiment focused on the effects of ANC on adolescent offspring which were born from prenatal stress mother. Second point focused on the effects of ANC on chronic stress-induced adult mice. Our study revealed ANC slowed the locomotor behavior both in adolescent offspring and adult mice as shown in total distance travelled and velocity reduction. Neurotransmitter measurement revealed ANC improves brain dopamine and gamma-aminobutyric acid (GABA) level accompanied with corticosterone reduction. The ANC attenuated brain inflammation by reducing interleukine-6 as well as increasing the IL-10 level. Our presentation would be detailed on effect of ANC in several affected tissue of stress model mice as well as molecular dynamic interaction of ANC in neurotransmitter receptors. Our finding would be emphasized the potential health roles ANC from local cultivar PSP in broad range inhibitor pathway to resist the impact on stress.

Keywords: antioxidant, antidepressant, BDNF, MAO enzyme, serotonin

S20-4

Probiotics as neuroplasticity agent for neurodevelopment and cognitive function in stunting

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Stunting or short stature still become a huge burden problem in Lower- and Middle-Income Countries (LMICs) giving it's short and long term consequences. Stunted toddler, aside from being physically short, have lower cognitive function compared to healthy ones. Since the discovery of Microbiota-Gut Brain Axis (MGB Axis) which interconnects gut microbiota and Central Nervous System (CNS), the intervention targeting gut microbiota becoming the promising non-invasive approach to treat various condition of CNS. The property of human CNS, called neuroplasticity, which allows the brain to rearrange itself both functionally and structurally, gives an additional information about possible mechanism in order to tackle the neurodevelopmental and cognitive impairment in stunting. Probiotics have been known to have the ability to protect gut health as well as brain conditions. Probiotics release substances called SCFAs which have positive impact on neuronal cells, as well as microglia, regulating BBB's integrity,

producing neurotropic factors (e.g. BDNF, GDNF), and promoting the formation of new synapse. Administration of certain probiotic, called *L. plantarum* has known to stimulate NOD2 signaling pattern in intestinal epithelial cells, promoting proliferation and production of IGF-1, which resulted in linear and CNS growth. These properties of probiotics made it become the promising and feasible treatment approach, especially in source-limited setting in LMICs.

Keywords: stunting, neuroplasticity, probiotics, neurodevelopment, cognitive impairment

S20-5

Translational regulation of centella asiatica-induced synaptic plasticity in neurodegenerative diseases predicted by network interaction

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Aging and age-related health issues, particularly neurodegenerative diseases, have grown as human life expectancy has increased. The expanding number of patients suffering from neurodegenerative diseases poses a substantial social and economic burden on society. Several neurodegenerative diseases are thought to be caused by an imbalance between reactive oxygen species and antioxidant enzymes, known as oxidative stress. Neuromodulation therapies that use both targeted neuroplasticity and synaptic plasticity to improve clinical outcomes in neurodegenerative disorders are being developed. The plasticity caused by activity modification leads to an increase in protein synthesis, which is regulated tightly at each stage of translation. Natural products are currently being employed as an alternative or integrative therapy agent because there is no cure. The aim of the study is to investigate how the mechanisms of translational regulation in Centella asiatica-induced synaptic plasticity predicted by Network Interaction work. We used the gene bank to get a variety of protein databases associated with synaptic plasticity. Cytoscape 3.9.1 was used to analyze the Protein-Protein Interaction (PPI). STITCH was used to predict Centella asiatica target constituents and their relationship with target synaptic plasticity, which was then followed by GO and KEGG pathway enrichment analysis. Ligands are then bound to presynaptic and postsynaptic receptors. The protein database yielded 446 protein-coding genes associated with synaptic plasticity. PPI and KEGG pathway analyses revealed the chemical could inhibit the Akt and mTORC1 pathways. TSC1, Rheb, FMRP, 4E-BP, and eIF4E were the proteins addressed. This study showed the potential of Centella asiatica in neurodegenerative disease by binding to various proteins, including TSC1, Rheb, FMRP, 4E-BP, and eIF4E. This Centella asiatica chemical has been identified to bind to the AKT1 and mTOR signaling pathways. Centella asiatica may be more effective in the treatment of neurodegenerative diseases. These findings point to a critical function for regulation of the eIF4F complex by 4E-BP in the initiation of translational mRNA in protein synthesis during synaptic plasticity.

Keywords: translational regulation, synaptic plasticity, centella asiatica, neurodegenerative diseases, network interaction

S21-1

The roles of STIM proteins in the cytosolic Ca^{2+} levels of skeletal muscle cells

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Skeletal muscle contracts or relaxes to maintain or change the body posture when standing still or during movements. For the contraction and relaxation of skeletal muscle, Ca^{2+} in the cytosol of skeletal muscle fibers is temporally and spatially regulated because the Ca^{2+} acts as a switch to turn on and off a series of contractile proteins by binding and unbinding to troponin C proteins. The cytosolic Ca^{2+} level in skeletal muscle fibers is governed mainly by two different Ca^{2+} pools: Ca^{2+} ions that are stored in the sarcoplasmic reticulum (SR) and Ca^{2+} ions in the extracellular space. Store-operated Ca^{2+} entry (SOCE) is a Ca^{2+} entryway from the extracellular space to the cytosol. Orai1 (a Ca^{2+} entry channel on the plasma/t-tubule membrane) and stromal interaction molecules 1 and 2 (STIM1 and STIM2, Ca^{2+} sensors on the SR membrane) are the main protein mediating SOCE in skeletal muscle. This talk focuses on the roles of STIMs along with SOCE in the physiological and pathophysiological functions of skeletal muscle and in their correlations with historical proteins that are known to mediate skeletal muscle function.

Keywords: STIM, SOCE, contraction, skeletal muscle, muscular dystrophy

S21-2

Reconstitution of skeletal muscle excitation-contraction coupling: Toward understanding of its molecular mechanism and related diseases

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In skeletal muscle excitation-contraction (E-C) coupling, depolarization of transverse tubule membrane causes conformational change in dihydropyridine receptor, which in turn opens type 1 ryanodine receptor (RyR1) to release massive Ca^{2+} from the sarcoplasmic reticulum. This 'depolarization-induced Ca^{2+} release' (DICR) occurs through a supramolecular complex composed of several proteins including RyR1, Cav1.1, β1a, Stac3, and junctophilin. However, it remains so far unclear about molecular mechanism of DICR, especially conformational change in RyR1. Recently, successful reconstitution of DICR was reported by co-expressing these essential components in nonmuscle cells (Perni et al., PNAS, 2017). We have improved the method to establish a high-throughput platform of reconstituted DICR in HEK293 cells. In this symposium, I will talk about establishment of the reconstituted DICR platform and its application to mutation validation and drug development for disease-causing mutations. I will also address recent progress in elucidation of molecular mechanism of DICR. Our reconstituted platform will accelerate understanding of mechanism of E-C coupling and the related diseases.

Keywords: ryanodine receptor, calcium release, calcium channel, skeletal muscle, drug development

S21-3

Characterization of type 1 ryanodine receptor knock-in mice with malignant hyperthermia-associated mutations

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The ryanodine receptor type 1 (RYR1) is a Ca^{2+} release channel located in the sarcoplasmic reticulum of skeletal muscle and plays an important role in excitation-contraction coupling. RYR1 is a giant homotetrameric protein complex with a large cytoplasmic structure, six transmembrane segments, and forms a cation channel. Mutations in the RYR1 gene cause severe muscle diseases such as malignant hyperthermia (MH), which is a disorder of Ca^{2+}-induced Ca^{2+} release via RYR1. To date, more than several hundred mutations of *RYR1* gene have been reported in MH patients. We have recently generated a novel MH mouse model (R2509C-RYR1 mice) using the CRISPR/Cas9 system. In R2509C-RYR1 heterozygous mice, MH-like episodes were induced by volatile anesthetics. In contrast, R2509C-RYR1 homozygous mice died in late embryonic stages. Furthermore, we are able to simultaneously measure $[Ca^{2+}]_{cyt}$ and cellular temperature and found that an increase in cellular temperature is associated with an increase in $[Ca^{2+}]_{cyt}$ upon application of isoflurane. In this symposium, I would like to present that the relationship between thermal signaling and Ca^{2+} homeostasis using this MH model mouse.

Keywords: calcium, skeletal muscle, ryanodine

S21-4

Structural basis for diamide modulation of ryanodine receptor

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Diamide insecticides, one of the top-selling insecticides, target insect ryanodine receptors (RyRs) and cause misregulation of calcium signaling in insect muscles and neurons. Several resistance mutations have been reported to reduce the efficacy of the diamides, but the exact binding sites and mechanism of resistance mutations are not clear. We solved the cryo-electron microscopy (cryo-EM) structure of RyR in complex with the anthranilic diamide chlorantraniliprole (CHL). CHL binds to the pseudo-voltage-sensor domain (pVSD) of RyR, a site in proximity to the previously identified resistance mutations. Mutagenesis studies in silico, in mutant cell lines, and in transgenic drosophila strains reveal the key residues involved in diamide coordination and the molecular mechanism under species-selectivity and resistance mutations. We have also systematically characterized several other diamide insecticides by functional and structural methods. Our results provide a foundation for developing novel pesticides to overcome the resistance crisis.

Keywords: ryanodine receptor, cryo-EM, diamide insecticide, resistance, selectivity

S22-1

Cryo-EM driven paradigm shift of ABC transporter mechanism

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Present in all kingdoms of life, ATP-binding cassette (ABC) transporters harness the energy of ATP binding and hydrolysis to translocate a multitude of chemically diverse substrates across cellular membranes. Despite decades of studies and many available structures, the molecular mechanisms of most ABC transporters are still poorly defined. The ongoing revolution of cryo-EM has enabled novel approaches for obtaining deep insights into these highly dynamic membrane protein machines. Through our cryo-EM studies of several ABC transporters that perform different functions, we have uncovered how distinct tasks of substrate translocation are accomplished by the unique actions of these transporters. Importantly, our own research experience in the past decade is an excellent demonstration of how single-particle cryo-EM methodology and the mechanistic study of ABC transporters stimulate each other's development, thus emphasizing the extremely versatile nature and yet-to-be-realized potential of cryo-EM in biological research.

Keywords: ATP-binding cassette, cryo-EM

S22-2

Elucidating the molecular architecture of the Gαi-bound TRPC5 ion channel

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G-protein coupled receptors (GPCRs) and ion channels play a pivotal role as molecular switches in transforming extracellular stimuli into intracellular effects. It has been postulated for a long time that ion channels act as direct effector molecules of the alpha subunit of G-proteins (Gα). However, the structural evidence supporting this direct interaction between Gα and ion channels is incomplete. In this talk, we present the cryo-electron microscopy structures of the human transient receptor potential canonical 5 (TRPC5)-$G\alpha_{i3}$ complexes with a 4:4 stoichiometry in lipid nanodiscs. Surprisingly, $G\alpha_{i3}$ binds to the ankyrin repeat edge of TRPC5 at a distance of approximately 50 Å from the cell membrane. The binding interface between the two proteins is composed of the Ile-Tyr-Tyr (IYY) motif of the ankyrin repeat domain (ARD) 1-2 of TRPC5 and α-helices 2 and 3 of $G\alpha_{i3}$. Our electrophysiological analysis further demonstrates that $G\alpha_{i3}$ is a direct activator of TRPC5, and both calcium and PIP2 are cofactors required for $G\alpha_{i3}$ to fully activate TRPC5. Intriguingly, $G\alpha_{i3}$ enhances the sensitivity of TRPC5 to PIP2, which makes TRPC5 more easily opened in the cell membrane, where the concentration of PIP2 is physiologically regulated. Our results provide a structural framework for elucidating the crosstalk between two major classes of transmembrane proteins: GPCRs and ion channels, and demonstrate that ion channels are among the direct effector molecules of Gα proteins triggered by GPCR activation.

Keywords: G protein-coupled receptors (GPCRs), transient receptor potential (TRP) channels, Gα, cryo-electron microscopy (cryo-EM)

S22-3

Structural basis of voltage-gated calcium channels

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Calcium ions (Ca^{2+}) play a crucial role in various physiological processes such as muscle contraction, neurotransmitter release, and cell signaling. Voltage-gated calcium channels (VGCCs) tightly regulate Ca^{2+} influx in response to membrane depolarization. VGCCs are classified into three main types: CaV1.x (L-type), CaV2.x (N, P/Q, R-type), and CaV3.x (T-type). T-type channels are distinct in their low membrane potential activation, fast gating kinetics, and small single-channel conductance. They are involved in numerous physiological processes and have been implicated in neurodegenerative and mental diseases.
Structural insights into T-type channels and their interactions with antagonists are essential for understanding drug selectivity and potency. Recent studies have reported the structures of CaV3.3 bound to non-selective CaV3 blockers, revealing overlapping binding sites. However, the differences in potency and selectivity of various CaV3 inhibitors cannot be easily explained by these binding sites. To investigate the basis of potent and selective T-type inhibitors, the structures of human CaV3.3 were determined using cryo-EM, either alone or bound to four different inhibitors. The results identified a new binding site for selective T-type pore blockers, revealed a common mechanism for selective inhibitors targeting different positions in CaV3 proteins, and uncovered the complex interplay between fenestrations, bound drugs, and lipids.

Keywords: calcium ions, voltage-gated calcium channels, non-selective CaV3 blockers

S22-4

Cryo-EM structure of a calcium-activate chloride channel BEST1 in a wide-open state

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Bestrophin-1 (BEST1) is a Ca^{2+}-activated chloride channel involved in various physiological processes including Ca^{2+} homeostasis, cell volume regulation, and gliotransmitter release. The structures of chicken BEST1 and human BEST1 (hBEST1) have shown that direct Ca^{2+}-binding leads to the opening of a narrow channel pore to transport Cl^-. However, these structures are not sufficient to provide structural insight to understand how BEST1 transports small monovalent Cl^- and large gliotransmitters. To understand ion transport mechanism of BEST1, we aimed to solve the molecular structure and characterize the ion permeability of hBEST1 channel. We have solved cryo-EM structures of hBEST1 in closed, intermediate, and wide-open conformations at the resolution of 2.42~2.98 Å. These structures provide three essential findings. First, hBEST1 undergoes large conformational changes of the transmembrane (TM) domain, which induces wider pore opening than previously suggested open conformation. In the wide-open structure, the whole TM helical bundles of each protomer are rotated ~32o in a rigid body movement instead of local rotation of the pore-lining helix, S2a. Second, multiple conformations visualize electron densities along the central axis of the pore, which may be permeant Cl^-. Third, a conserved Gln, Q208 in the cytoplasmic domain coordinates a presumably Cl^-. Indeed, Q208 mutations showed altered current density as well as anion selectivity, especially for large organic anions. Currently, we are examining the electrophysiological characteristics of mutant channels inspired by cryo-EM structures.

Keywords: bestrophin, Ca^{2+}-activated chloride channel, cryo-EM, gating

S22-5

Conformational changes in the human Cx43/GJA1 gap junction channel visualized using cryo-EM

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Connexin family proteins assemble into hexameric hemichannels in the cell membrane. The hemichannels dock together between two adjacent membranes to form gap junction intercellular channels (GJIChs). We report the cryo-electron microscopy structures of Cx43 GJICh, revealing the dynamic equilibrium state of various channel conformations in detergents and lipid nanodiscs. We identify three different N-terminal helix conformations of Cx43—gate-covering (GCN), pore-lining (PLN), and flexible intermediate (FIN)—that are randomly distributed in purified GJICh particles. The conformational equilibrium shifts to GCN by cholesteryl hemisuccinates and to PLN by C-terminal truncations and at varying pH. While GJIChs that mainly comprise GCN protomers are occluded by lipids, those containing conformationally heterogeneous protomers show markedly different pore sizes. We observe an α-to-π-helix transition in the first transmembrane helix, which creates a side opening to the membrane in the FIN and PLN conformations. This study provides basic structural information to understand the mechanisms of action and regulation of Cx43 GJICh.

Keywords: gap junction, connexin 43, gating, cryo-EM, structure

S23-1

Modulation of neural circuit properties by synaptic suppressors

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Synapses are fundamental information units of the brain that function by establishing and regulating innumerable overlapping and inter-digitating neural circuits between neurons. Synaptic cell-adhesion molecules (CAMs) are central synapse organizers that structurally align pre- and postsynaptic membranes and functionally coordinate assembly of pre- and postsynaptic machineries that are essential for instructing cell-type specificity, neuronal specification, and the diversity of individual synapse functions. My laboratory has spent recent years identifying key synaptic CAMs and studying their mechanisms in shaping distinct synaptic signaling pathways. Our hypothesis is that the number, location, and properties of diverse synapses are determined by interactions between pre- and postsynaptic CAMs and their associated signaling molecules, and we refer to the rules by which the network of these proteins build neural circuits as the molecular logic of neural circuit architecture. In this talk, I will discuss our recent studies on modulation of *trans*-synaptic mechanisms tuned by a specific class of membrane-anchored proteins and touch on potential implications not only for understanding how neural circuits are designed, but

also how brain disorders might be driven, at least in part, by synaptic impairments.

Keywords: synapse, neural circuit, synaptic inhibition, synaptic suppressor

S23-2

Neural circuit of orexin-induced hyperphagia

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Orexin (or hypocretin) is a hypothalamic neuropeptide that regulates wakefulness and appetite. While it was initially suggested that orexin promotes feeding by the hypocretin receptor (*Hcrtr*) expressed by hypothalamic neurons, the identity of responsible neural circuits still remain to be identified. In particular, *Hcrtr2* is highly expressed by the anorexigenic pro-opiomelanocortin (POMC) neurons, but the role of *Hcrtr2* expressed by POMC neurons remains unclear. In this study, we investigated the neural mechanisms for orexin-induced hyperphagia. We used multiple approaches including patch-clamp electrophysiology, immunohistochemistry, and *in vivo* feeding studies to gain insight into the neuronal circuits responsible for the orexigenic effects of orexin A. We found that applications of orexin A directly depolarize a distinct subpopulation of POMC neurons. In addition, *in vivo* experiments demonstrated that *Hcrtr2* expressed by the POMC neurons is responsible for the orexigenic effects of orexin A. We also identified the neural circuit downstream of POMC neurons. Together, our findings demonstrate that orexin A increases food intake via the activation of arcuate POMC neurons and the downstream neural circuits.

Keywords: hypothalamus, POMC neurons, phospholipase C beta, opioid receptor, mouse genetics

S23-3

Thalamocortical network dynamics underlying perceptual inference

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Although information that reaches the brain from the senses is often ambiguous and disconnected, our perception of the world (internal model) is stable and continuous. The ability to maintain stable perception of the world despite the disconnected and ambiguous information received from the senses suggests that the brain supplements immediate inputs with patterns extracted across previous stimuli to create an internal model that can then guide behavior. Although how such an internal model could be created and updated remains poorly understood, previous investigations suggest that "higher-order" thalamic nuclei might play a key role by selectively stabilizing short-term representations (Schmitt et al. 2017). Here I present electrophysiological and optogenetic results demonstrating that thalamocortical interactions between the posterior parietal cortex (PPC) and its' thalamic counterpart, the pulvinar (PUL) are necessary for maintenance and updating of short-term representations that underlie perceptual inference. Multi-area recordings of single-unit spiking activity show that cooperative interactions between these thalamic and cortical circuits encode patterns across previously encountered stimuli that are used to make decisions when current inputs are ambiguous. We further find that maintenance and updating of the representation used for inference likely involves thalamic recruitment of inhibitory populations, in part by controlling updating of PPC representations. In addition to identifying a novel circuit interaction involved in inference-based decision-making, these findings suggest mechanisms by which thalamocortical interactions may be broadly relevant to short-term maintenance of representations in the brain.

Keywords: thalamocortical, inference, decision-making, multi-area recording, circuit dynamics

S23-4

Opposite-directional activity of posterior parietal cortex neurons in erroneous decision-making during delayed match-to-sample task

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Making correct and timely decisions in a complex and dynamic environment is a significant challenge for the brain, and inaccurate decisions are made with a certain frequency. Studies suggest that the activities in the posterior parietal cortex (PPC) represent the accumulation of evidence during perceptual decision-making. Here, we hypothesized that inaccurate representation of visual stimuli or decay of the correct representation of accurate visual stimuli would be observed in erroneous decision-making. To test this, we measured neuronal activity of the PPC using in vivo two-photon Ca^{2+} imaging in head-restrained mice performing a visual stimuli-based delayed two-alternative decision-making task. Using a new method based on the generalized linear model, we sorted 2867 neurons out of 5548 that showed epoch-specific activation. Approximately 36.4% of the PPC neurons showed a selective increase in firing frequency in an epoch-selective manner, 30.5% showed sensory stimuli-dependence, and 27.1% showed choice-dependent firing patterns. We found that the activities of a considerable number of neurons were associated with the opposite direction in error trials, including sensory-related (15.1%) and choice-related (13.6%) neurons in the PPC. Our results suggest that the activity of PPC neurons represents the directions of sensory stimulus and motor plan, and mis-assigned activities can lead to erroneous decision-making. We are currently examining the population encoding of PPC neurons to account for choice in both correct and erroneous decision-making.

Keywords: posterior parietal cortex, calcium imaging, error trials, decision making

S18-1

Collaborative approach of basic-clinical teachers in teaching during different organ-system based curriculum models

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The curriculum system based on organ system integration is in line with the cognitive characteristics of students and the laws of medical education, which is conducive to improving the quality of public health services. Therefore, its application is becoming increasingly widespread. The course system of organ system integration can be simply divided into two modes based on the different stages of

integration: interdisciplinary integration within the subjects of basic medical sciences and integration of the subjects between basic medical sciences and clinical medicine.
The factors that affect the implementation effect of the integrated curriculum system include course goal setting, course content arrangement, teaching method innovation, assessment and evaluation methods, student investment analysis, teacher teaching ability, feedback closed-loop improvement, etc. One of the most influential links is the training and improvement of teachers' teaching abilities, and the cooperation between basic and clinical teachers in different integrated systems is particularly crucial.
Collaboration between the basic and clinical teachers is critical for the successful medical education. In the organ-system based curriculum in Peking University, there is extensive collaboration between the teachers in basic medical sciences and clinical medicine. We will give a detail information during the conference.

Keywords: organ system, integration, basic medical sciences, clinical medicine, collaboration

S18-2

Integration of basic-clinical physiology using vignette questions for assessment of Indonesian student

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Appropriate use of assessment can properly test students' knowledge and skills. The aim of an assessment is to test the understanding of the learning objectives, and choosing the correct assessment method is important in determining this. Assessment can also be an influential dynamo, in which students become more motivated and eager to learn to reach their goals on assessment. Because assessment have such a powerful influence on student learning, it is important to develop a comprehensive assessment method that is able to analyse the understanding of several concepts by using one problem that accurately align with the learning objectives. Multiple-choice questions (MCQs) enable for the evaluation of knowledge comprehension and application across multiple topics. The large number of questions that can be posed in a single exam and the fact that a large number of students can take the exam simultaneously encourage the pervasive use of exams administered using this method. However, in order for the MCQ to be able to evaluate students' ability to apply knowledge and not just evaluate memorization (recall), the items need to be well structured. In physiology, understanding of conceptual knowledge, cause and effect, and critical thinking is imperative. Thus the purpose of asking the application of knowledge requires contextual MCQ items; in this case related to clinical cases in either individuals, groups or families and communities. In medical faculties across Indonesia, MCQ is used as one of the methods to evaluate students' knowledge application along their education years and also in their final national competency board exam (Uji Kompetensi Mahasiswa Program Profesi Dokter/UKMPPD). To ensure that the questions administered in this high stake examination is of high quality, a thorough process of preparation, review, and analysis of MCQ items are performed to reduce the influence of 'guessing' abilities and unnecessary difficulties.

Keywords: assessment, medical education, vignette questions, basic-clinical physiology, MCQs

S18-3

The practice of exposure to simulated and real patients along with doctor patient role play in CBL and PBL tools for horizontal and vertical integration at the University College of Medicine and Dentistry (UCMD), The University of Lahore (UOL), Lahore, Pakistan

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Background: UCMD is one of the pioneers of the integrated modular curriculum in the Punjab province of Pakistan. We expose our students to patients from their very first year. However, for patient safety and learning of communication, examination skills, and students engagement, we encourage and guide our students to assume the role of simulated patients or project video and investigations of real patients and present it as a trigger for Case Based Learning (CBL) and Problem-Based Learning (PBL).
Methodology: First-year medical students are exposed to both CBL and PBL in small groups consisting of 25 members. The peer-reviewed CBL scenarios and their learning outcomes are created and uploaded a week before the session. Students observe real patients in the ward or on YouTube videos and gather information from the text and reference books. They choose role players within their group and practice to perform as simulated patients, attendants, and doctors under supervision. It creates an engaging stimulus in comparison with a boring paper case and helps in the contribution of teaching and learning resource material for future use and critical reflection. Likewise, the first peer-reviewed trigger of PBL is uploaded a week before the session. Students prepare similarly for doctor-patient role-play. The remaining triggers to narrow down the diagnosis through examination and investigation findings are shared gradually during the session so that students can brainstorm after deriving their own learning outcomes. CBL is conducted in one session but PBL is in two sessions at a gap of one week.
Results: Students develop a visual picture of the patient presentation. Due to performance within each batch and the use of the local language of the simulated patient, they learn how to approach the patient and understand the terms typically used to explain the symptoms by the patients in our contextual situation. They feel confident in the history taking of a real patient in the ward after practicing on a simulated patient who is their own batch-fellow. At the end of CBL and PBL critical reflection by students and debriefing by facilitator further enhance learning.
Conclusion: Students become critical thinkers equipped with communication skills by these techniques which also create a friendly but professionally safe learning environment.

Keywords: PBL, CBL, role-play, simulated-patient, students-engagement

S18-4

Enhancing basic-clinical medical sciences integration while retaining the core principle of physiology in organ-system based curriculum

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In recent years, many medical schools worldwide have adopted the organ-system based curriculum to integrate basic sciences and clinical sciences. The integration of physiology teaching into the organ-system based curriculum has many advantages; for example, students are able to learn anatomy, biochemistry, pharmacology, pathology and physiology within the organ system simultaneously. Combining basic sciences such as anatomy, biochemistry, pharmacology, pathology, etc., into the curriculum allows medical students to integrate the relevant knowledge and build a comprehensive understanding of the human body. Compared with the traditional subject-based curriculum, the organ-system based curriculum also provides medical students the opportunity for early exposure to clinical scenarios. The close connection of physiological knowledge with clinical diseases in teaching can rectify the shortcomings of impracticality in traditional subject-based curriculum, allowing medical students to link pathophysiology with clinical cases during learning physiology immediately. On the other hand, physiology is, by principle, a logically rigorous subject that emphasizes cause-and-effect relationships. However, in the organ-system based curriculum, the original links between concepts are disconnected and scattered across different organ system sessions, thus losing the intrinsic coherence of physiology as an independent discipline. This way, medical students' overall understanding of physiology may be reduced. To address these problems, it is necessary to organize the curriculum carefully to maintain the necessary proportion of physiology in the organ-system based curriculum. In addition, innovative teaching methods such as self-directed learning and flipped classrooms should be used to integrate physiological knowledge with clinical applications. Teachers change their role from knowledge deliverers to facilitators of information integration. This can be achieved through the use of simulation software to simulate physiological responses of the human body in health and disease states and through the use of various pedagogical methods such as problem-based learning, team-based learning, and case studies to stimulate critical thinking and to facilitate the application of physiological principles to clinical problems. The ultimate aim is to enhance basic-clinical sciences integration while retaining the core principle of physiology in organ-system based curriculum.

Keywords: Basic-Clinical Medical Sciences Integration, Organ-System Based Curriculum, Subject-Based Curriculum, Self-Directed Learning, Flipped Classroom

S18-5

Collaborative approach of basic and clinical medicine in teaching of clinical presentation and pathophysiology

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When a physician sees a patient, the first thing they hear from the patient is the chief complaint. Based on the chief complaint, additional tests such as history-taking, physical examination, imaging, blood tests, and urine tests are performed to arrive at a final diagnosis. Inje University College of Medicine is currently conducting a course called "Clinical Presentation and Pathophysiology" in the context of basic medicine and clinical medicine, focusing on understanding the pathophysiology of various clinical presentations during each class session. The course is centered around five clinical presentations: weight loss, acute diarrhea, shock, cough, and acute abdominal pain.

Students watch video cases and create concept maps related to the clinical presentation in small groups. They are required to write SNAP (Summary, Narrowing, Analyze, Plan) reports, and present their concept maps and SNAP reports for evaluation and feedback from other small groups as well as teachers. Finally, there is a one-hour joint lecture given by teachers from the fields of basic medicine and clinical medicine. During this lecture, the basic medicine covers normal physiology and pathophysiology related to clinical presentation, while the clinical medicine discusses the principles of diagnosis and treatment in real clinical scenarios.

Through this process, students train in thinking about how physiological and pathophysiological principles apply to clinical presentations and how to utilize them in diagnosis and treatment. Feedback from both students and participating teachers on this process will also be introduced.

Keywords: clinical presentation, pathophysiology, concept map, basic medicine, clinical medicine

S39-1

Correlates of social behavior in cerebellum and anterior cingulate cortex

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The cerebellum has been implicated in the regulation of social behavior. Its influence is thought to arise from communication, via the thalamus, to forebrain regions integral in the expression of social interactions, including the anterior cingulate cortex (ACC). However, the signals encoded between the cerebellum and these brain regions is poorly understood. Here, we developed the E-Scope, an electrophysiology-integrated miniature microscope, to synchronously measure extracellular electrical activity in the cerebellum along with calcium imaging of the ACC. This single coaxial cable device combined these data streams to provide a powerful tool to monitor the activity of distant brain regions in freely behaving animals. During social behavior, we recorded the spike timing of multiple single units in cerebellar right Crus I (RCrus I) Purkinje cells (PCs) or dentate nucleus (DN) neurons while synchronously imaging calcium activity in contralateral ACC neurons. We found that during social interactions a significant subpopulation of cerebellar PCs were strongly inhibited, while most modulated neurons in the DN were activated. As expected, we find that there are higher correlations in the activity of cerebellar and ACC neurons that are similarly excited or inhibited by social interaction than in the activity of those modulated in an opposing manner. Surprisingly, these distinctions in correlations largely disappeared when only non-social epochs were analyzed suggesting that cerebellar-cortical interactions were behaviorally specific. Our work provides new insights into the complexity of cerebellar activation and co-modulation of the ACC during social behavior and a valuable open-source tool for simultaneous, multimodal recordings in freely behaving mice.

Keywords: imaging, electrophysiology, social, cerebellum, prefrontal

S39-2

Cerebellum as a crucial component in the regulation of depression-like behaviors

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The cerebellum has been traditionally considered to work for motor coordination and learning by receiving and processing sensorimotor information. As the cerebellar contributions to non-motor cognitive functions and mental controls have been increasingly recognized, the cerebellum likely receives and processes other types of information, and accordingly sends output signals to other brain regions. We have tested the cerebellar involvement in the chronic stress-mediated depression-like behaviors in mice. We found that chronic stress application triggering depression-like behavior led to an increase in c-Fos expression in deep cerebellar nucleus (DCN) neurons of the dentate nucleus (DN), indicating an activity increase in these neurons by chronic stress application. The chemogenetic inhibition of the DCN neurons that specifically project to the ventral tegmental area (VTA) during chronic stress application prevented mice from showing stress-dependent behavioral alterations. Oppositely, chronic excitation of these DCN neurons alone was capable of triggering behavioral alterations that are similar to stress-dependent depression-like behaviors. Based on these results, we concluded that the cerebellum directly regulates the development of stress-dependent depression-like behaviors, through the activity increase in specific DCN neurons in the DN that project to the VTA. Thus, the cerebellum appears to be involved in the regulation of stress responses according to the environmental situations. Two subsequent questions would then naturally arise: how DCN neurons are activated by stress application, and how the chronic activation of DCN neurons projecting to the VTA leads to the depression-like behaviors. In this symposium, I introduce our recent studies on these questions.

Keywords: cerebellum, depression, chronic mild stress, ventral tegmental area, plasticity

S39-3

Neurobiological imaging of neuropathic pain using PET

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Chronic neuropathic pain represents a serious worldwide health problem leading to life-long treatment and the possibility of significant disability. And it remains refractory to treatment despite a variety of therapeutic approaches. However, objective differentiation and diversity of neuropathic pain in humans which is based on patients' symptom descriptions is a persisting challenge.
Non-invasive human brain imaging using PET or MRI in chronic pain research led to the understanding that chronic pain patients display brain structure and function alterations, which provide complementary information for brain mechanisms underlying neuropathic pain. [^{18}F] FDG-PET imaging studies which show the overall regional metabolic changes in the brain are commonly conducted to evaluate the underlying neural mechanisms in patients with chronic pain after traumatic spinal cord injury or trigeminal neuralgia. The development of neurochemical-based PET imaging such as mGluR5 or GABA receptor PET or neuroinflammation imaging can allow the further understanding of the neural mechanism of neuropathic pain, and in particular, the therapeutic modulation.
In this session, we will review the in-vivo human imaging performed so far in chronic pain patients, which could help in expanding our understanding of diverse chronic pain in humans.

Keywords: chronic neuropathic pain, Positron emission tomography, FDG PET, mGluR5

S39-4

Intrinsic plasticity of Purkinje cell serves homeostatic regulation of fear memory

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Two forms of plasticity, synaptic and intrinsic, are neural substrates for learning and memory. Abnormalities in homeostatic plasticity cause severe neuropsychiatric diseases, such as schizophrenia, autism, and Alzheimer's disease. This suggests that the balance between synaptic transmission and intrinsic excitability is important for physiological function in the brain. While previous studies have revealed that the synapses between parallel fibers (PFs) and Purkinje cells (PCs) are potentiated following auditory fear conditioning (AFC), little is known about intrinsic plasticity in PCs in fear memory processing. We propose intrinsic plasticity as a novel mechanism of fear memory in PCs and investigate its interaction with previous findings. We used electrophysiology and immunoblotting to investigate neuronal plasticity in PCs following AFC. We utilized pharmacology and optogenetics to manipulate molecular changes and intrinsic plasticity and analyzed fear-related behavioral changes in male mice. Intrinsic excitability of PCs decreased following AFC via upregulation of small-conductance Ca^{2+}-activated K^+ channel type 2 (SK2 channel). Depressed excitability served as homeostatic regulation to balance PF-evoked PC firing. Pharmacological blockade of the SK2 channel following conditioning induced excessive fear memory. Furthermore, optogenetic manipulation revealed that abnormal activity of PCs during the early consolidation period bidirectionally controls fear memory. The findings suggest that information related to fear memory is modified by synaptic potentiation—but counterbalanced by decreased intrinsic excitability to maintain stable PC activity and sustain fear memory in a normal range.

Keywords: intrinsic plasticity, purkinje cell, auditory fear conditioning, homeostatic regulation, optogenetics

S25-1

Targeting senescent cells for the treatment of age-associated disease

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Epidemiological studies have shown that age is the dominant risk factor for lifestyle-related diseases. The incidence and the prevalence of diabetes, heart failure, coronary heart disease and hypertension increase with advancing age. However, the molecular mechanisms underlying the increased risk of such diseases that is conferred by aging remain unclear. Cellular senescence is originally described as the finite replicative lifespan of human somatic cells in culture. Cellular senescence is accompanied by a specific set of phenotypic changes in morphology and gene expression including negative regulators of the cell cycle such as p53. Primary cultured cells from patients with premature aging syndromes are known to have a shorter lifespan than cells from age-matched healthy persons. It is also reported that the number of senescent cells increases in various tissues with advancing age. Interestingly, such accumulation of senescent cells in aged animals is attenuated by caloric restriction that regulates the lifespan regulatory system and delays age-associated phenotypes. I therefore hypothesize that cellular senescence in vivo contributes to the pathogenesis of age-associated disease and have shown a critical role of cellular senescence in age-related pathologies. However, a direct inhibition of cellular aging signaling would lead to the increased incidence of cancer, so we need to develop anti-senescent therapy without cancer development. Here I will show our recent data on a novel strategy of anti-senescent therapy for age-associated disease by targeting cellular senescence (Seno-antigens, Seno-anergy-related molecules), which would not promote tumorigenesis.

Keywords: cellular senescence, seno-antigen, seno-anergy

S25-2

TFEB activation as a response to lysosomal stress in obesity or metabolic syndrome- Role of TFEB activation as a potential measure against senescence -

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Senescence is observed in pancreatic b-cells, adipocytes, T cells, and hepatocytes of diverse metabolic diseases such as obesity, diabetes or NASH, which is likely to play a significant role of the development of such diseases. Senescence is also closely associated with dysregulated autophagy and lysosomal stress/dysfunction. We recently observed features of lysosomal dysfunction and senescence in adipocyte macrophages (ATMs) of obese subjects or experimental animals. Thus, lipofuscin accumulation and SA-b-gal activity were observed in ATMs of obese subjects or mice. *Tfeb*, a key regulator of lysosomal biogenesis and autophagy, and TFEB-downstream genes were also induced in ATMs of obese subjects or mice, likely due to organelle stress such as lysosomal stress. To unravel the significance of these findings, we generated macrophage-specific *TFEB*-overexpressing mice and observed complete abrogation of diet-induced obesity, adipose tissue inflammation and insulin resistance, which was independent of autophagy but dependent on *TFEB*-induced GDF15 expression. Palmitic acid induced *Gdf15* expression through lysosomal Ca^{2+}-mediated TFEB nuclear translocation in response to lysosomal stress. In contrast, high-fat diet-fed mice with macrophage-specific *Tfeb* deletion showed aggravated adipose tissue inflammation and insulin resistance, accompanied by reduced GDF15 level. Finally, we also observed activation of TFEB-GDF15 axis in ATMs of obese human subjects as a consequence of lysosomal stress. These findings suggest importance of TFEB in lysosomal stress response associated with obesity or metabolic syndrome, and also as a promising therapeutic target for treatment of these conditions. TFEB activators might be able to ameliorate senescence-associated features or deranged metabolic profile in metabolic diseases such as obesity, metabolic syndrome or NASH.

Keywords: lysosome, autophagy, obesity, diabetes, macrophages

S25-3

Progression of cellular senescence and immunosenescence in non-alcoholic steatohepatitis

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The prevalence of Non-alcoholic fatty liver disease (NAFLD) has been increasing steadily, and non-alcoholic steatosis hepatitis (NASH), a severe form of NAFLD characterized by hepatic inflammation with fibrosis, is becoming more common. NASH can even lead to hepatocellular carcinoma without the presence of cirrhosis, highlighting its clinical significance severity. Multiple evidence support that cellular senescence, defined as irreversible cell cycle arrest caused by various processes that render viable cells dysfunctional, hampering normal tissue homeostasis, is considered as one of essential mechanism on age-related diseases including NAFLD. As there is no FDA approved medications for treating NASH, it is crucial to understand pathophysiologic mechanisms between parenchymal and non-parenchymal cells in the liver, focusing on senescence. In this study, we developed a novel human-like NASH mouse model and investigated characteristics of senescence in hepatocytes and immunosenescence in non-parenchymal cells such as T cells and myeloid cells.
C57BL/6J mice were fed a fructose-fat enriched, no choline (FFEN) diet for 4, 8, 12, 16, and 20 weeks. As the duration of the FFEN diet increased, surrogate markers of liver injury such as serum ALT and AST levels, liver weight, lipid droplet accumulation, liver inflammation, and fibrosis gradually increased in the NASH mice model. NASH mice showed cellular senescence, as indicated by senescence-associated (SA)-β galactosidase staining, and early upregulation of mRNA expression of senescence markers (p21, p16), pro-inflammatory and fibrosis markers, as measured by RT-qPCR. We also examined changes in the immune cell population using flow cytometry analysis. After 20 weeks, NASH mice exhibited a significant increase in $CD8^+$ T cells compared to $CD4^+$ T cells. The exhaustion marker PD-1 was upregulated in activated T cells ($CD44^+CD4^+$ or $CD8^+$) in NASH mice even after 4 weeks of FFEN diet. Exhaustion related key transcription factors, Tox and Eomes were significantly increased in exhausted T cells ($PD\text{-}1^+CD4^+$ or $CD8^+$). Furthermore, liver resident macrophages, known as Kupffer cells ($CD11b^{int}F4/80^{high}Clec4F^{high}$), decreased, while monocyte-derived macrophages ($CD11b^{high}F4/80^{low}$) and inflammatory monocytes ($CD11b^+Ly6C^{high}$) increased in a time-dependent manner in NASH mice. In conclusion, our study demonstrated that cellular senescence and immune cell transition were observed in the early stages of NASH using a NASH mouse model induced by the novel FFEN diet.

Keywords: non-alcoholic steatosis hepatitis, cellular senescence, immunosenescence, T cell, macrophage

S25-4

Adipose tissue senescence in insulin resistance

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Adipose tissue is one of the most vulnerable tissues to aging. Adipose tissue aging promotes low-grade inflammation, fibrosis, and insulin resistance and affects other peripheral tissues by releasing inflammatory cytokines. Obesity not only increases lipid accumulation in peripheral tissues but also accelerates tissue aging, including adipose tissue. The adipose tissue of obese subjects demonstrates the increased number of senescent cells, which contributes to the development of tissue dysfunction and aging phenotype. Therefore, killing the senescent cells (senolytics) or reversing the senescent cells to young cells (senomorphics) can delay tissue aging and aging-associated phenotype in adipose tissue. Recently, eliminating the senescent cells in adipose tissue attenuates insulin resistance in high-fat diet (HFD)-fed obese mice, suggesting that targeting cellular senescence in adipose tissue could be the potential treatment for type 2 diabetes. Currently, however, there are no clinically available senotherapeutics. We aimed to find novel senotherapeutics for adipose tissue aging and insulin resistance.We tested 2,150 clinically applicable compounds for their senotherapeutic effects in senescent human dermal fibroblast (HDF) by using cell toxicity or senescent-associated beta-galactosidase (SaβGal) staining. Ten compounds, which have senotherapeutic effects in HDF cells, were examined for their effect on glucose metabolism in HFD-fed obese mice using a glucose tolerance test. Finally, we found one compound (HT) attenuating insulin resistance and adipose tissue senescence in obese mice.The newly found senotherapeutic compound reduced weight gain and fat mass but had no effect on food intake in HFD-fed obese mice. It reduced SaβGal staining in adipose tissue both in obese mice and aged mice, which was followed by a reduction in large-sized adipocytes and the number of crown-like structures. HT improved glycemic control and insulin resistance both in obese mice and aged mice. It also attenuated adipose tissue senescence in human subcutaneous adipose tissue ex vivo. It also demonstrated delayed aging phenotype and extended life span in mice. Thus, these results suggest that we find a novel senotherapeutic agent that may be a potential therapeutic for type 2 diabetes.

Keywords: senescence, adipose tissue, senotherapeutics, obesity

S26-1

PRRX1 is a master transcription factor of stromal fibroblasts for myofibroblastic lineage progression

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Although stromal fibroblasts play a critical role in cancer progression, their identities remain unclear as they exhibit high heterogeneity and plasticity. Here, a master transcription factor (mTF) constructing core-regulatory circuitry, *PRRX1*, which determines the fibroblast lineage with a myofibroblastic phenotype, is identified for the fibroblast subgroup. *PRRX1* orchestrates the functional drift of fibroblasts into myofibroblastic phenotype via TGF-β signaling by remodeling a super-enhancer landscape. Such reprogrammed fibroblasts have myofibroblastic functions resulting in markedly enhanced tumorigenicity and aggressiveness of cancer. PRRX1 expression in cancer-associated fibroblast (CAF) has an unfavorable prognosis in multiple cancer types. Fibroblast-specific *PRRX1* depletion induces long-term and sustained complete remission of chemotherapy-resistant cancer in genetically engineered mice models. This study reveals CAF subpopulations based on super-enhancer profiles including *PRRX1*. Therefore, mTFs, including *PRRX1*, provide another opportunity for establishing a hierarchical classification system of fibroblasts and cancer treatment by targeting fibroblasts.

Keywords: PRRX1

S26-2

Crustacean ECM derived miRNAs induce tumor cell death

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Micro RNA of Marsupenaeus japonicas has been known to promote apoptosis of tumor cells. However, the detailed mechanisms are not well understood.
Using tomographic microscope, which can detect the internal structure of cells, we observed breast tumor cells following treatment of the miRNA. Intriguingly, we found that mitochondria migrate to an adjacent tumor cells through a tunneling nanotube. To recapitulate this process, we engineered a microfluidic device through which mitochondria were transferred. We show that this mitochondrial transfer process released endonuclease G into tumor cells, which we referred to herein as unsealed mitochondria. Moreover, unsealed mitochondria had synergistic apoptotic effect with subtoxic dose of doxorubicin thereby mitigating cardiotoxicity.
Together, we show that the mitochondrial transfer through microfluidics can prove potential novel strategies towards tumor cell death.

Keywords: micro RNA, ECM, mitochondria, tomographic microscope, microfluidic device

S26-3

Proteome of decellularized human colorectal tissues reveals a mesenchymal hallmark of the cancer-associated extracellular matrix

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Stromal heterogeneity influences the efficiency of adjuvant therapy in colorectal cancer (CRC). Few studies have examined the relationship between stromal remodeling and extracellular matrix (ECM) changes in CRC. Using multiplexed isobaric tandem mass tag (TMT) tagging, we characterized the ECM composition of decellularized human colorectal cancer tissues. By merging single-cell transcriptome data from CRC patients, we uncovered the cellular origin leading to cancer-related ECM change. 18 tumor-enriched proteins are mainly produced by tumor fibroblasts, whereas 20 tumor-depleted proteins are produced by normal fibroblasts. Among 29 mesenchymal CRC subtype-enriched genes showing favorable correlations with the gene sets of transforming growth factor beta response and epithelial-mesenchymal transition, 10 matrisome genes, composed of tumor-enriched type XI

collagen alpha 1 chain (*COL11A1*), collagen triple helix repeat containing 1 (*CTHRC1*), and thrombospondin 2, in addition to normal-enriched mimecan (*OGN*), tenascin-X (*TNXB*), and SPARC-like protein-1 (*SPARCL1*), were correlated with unfavorable prognosis in patients with colorectal cancer. Our ECM-focused profiling of tumor stroma may reveal new insights into cellular mechanisms governing the matrix-based cancer development and could serve as indicators for biological processes and clinical endpoints.

Keywords: extracellular matrix, colorectal cancer, matrisome, stroma heterogeneity, EMT

S26-4

Cell dynamics in the omentum: Insights into metastasis prevention

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The omentum is a fatty tissue that extends from the stomach, and contains immune cell structures called milky spots that filter pathogens in the peritoneal cavity. However, ovarian and gastrointestinal cancer cells that circulate in the peritoneal fluid can be trapped in milky spots and form implants. It is difficult to remove the omentum completely, and there are no effective strategies to prevent occult cancer cells in the peritoneal fluid from implanting onto preserved omentum tissues. Studies by my laboratory have shed insights into pre-metastatic niche formation in the omentum, and how the risk of metastasis might be minimized. We recently discovered that normal saline, which is commonly administered into the peritoneal cavity for diagnostic and intra-operative lavage, is prominently absorbed by the omentum and exfoliates its mesothelial lining. This in turn stimulated recruitment of a subpopulation of monocyte/macrophages that promoted neoangiogenesis and cancer cell implantation in milky spots. By contrast, these deleterious effects were not observed when a biocompatible lavage solution was used. Our findings raise the possibility that normal saline stimulates the receptivity of the omentum for cancer cells, and that the risk of metastasis can be minimized by using a biocompatible solution for lavage procedures.

Keywords: metastasis, omentum, pre-metastatic niche

S27-1

The cardiac vagus has a vital role in maintaining coronary artery blood flow during exercise

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The phrase 'complete vagal withdrawal' is often used when discussing autonomic control of the heart during exercise. Evidence for vagal withdrawal during exercise comes from historic studies examining heart rate control and the use of cholinergic blockers. However, more recent studies have challenged this assumption. We hypothesized that cardiac vagal activity increases during exercise and plays a fundamental role in maintaining cardiac function mediated by transmitters other than acetylcholine.
Chronic direct recordings of cardiac vagal nerve activity (CVNA), cardiac output, coronary artery blood flow and heart rate were recorded in conscious adult sheep (n = 6 - 9) during whole-body treadmill exercise. Cardiac innervation of the left cardiac vagal branch was confirmed with lipophilic tracer dyes (DiO). Sheep were exercised with pharmacological blockers of acetylcholine (atropine, 250 mg), vasoactive intestinal peptide (VIP) ([4Cl-D-Phe6,Leu17]-VIP 25 µg), or saline control, randomized on different days. In a subset of sheep, the left cardiac vagal branch was denervated.
Neural innervation from the cardiac vagal branch is seen at major cardiac ganglionic plexi and within the fat pads associated with the coronary arteries. Directly recorded CVNA increased during exercise. Left cardiac vagal branch, denervation attenuated the maximum changes in coronary artery blood flow (maximum exercise, control: 63.5 ± 5.9 ml/min, cardiac vagal denervated: 32.7 ± 5.6 ml/min. $P<0.001$), cardiac output and heart rate during exercise. Atropine did not affect any cardiac parameters during exercise, but VIP antagonism significantly reduced coronary artery blood flow during exercise to a similar level to vagal denervation.
Our study challenges the conventional view and demonstrates that cardiac vagal nerve activity actually increases and is crucial for maintaining cardiac function during exercise. Furthermore, our findings show the dynamic modulation of coronary artery blood flow during exercise is mediated by VIP and that acetylcholine has no role in modulating cardiac function during exercise.

Keywords: vagal nerve, coronary blood flow, exercise, autonomic nervous system, co-transmitter

S27-2

Vagus nerve stimulation and its cardioprotective effects against myocardial ischemia/reperfusion injury

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Several cardiovascular diseases are accompanied by imbalance of the autonomic nervous system, specifically decreased parasympathetic activity. In patients with acute myocardial infarction, the same type of imbalance has been shown to associate with increased risk for cardiac mortality. Over the past several years, enhancing parasympathetic activity by delivering electrical stimulation to the cervical vagus nerve has emerged as a promising therapy for various conditions. Experimental studies have demonstrated that vagus nerve stimulation (VNS) exerted cardioprotection against cardiac ischemia/reperfusion (I/R) injury. However, whether the cardioprotection of VNS are mainly due to direct vagal activation through its efferent fibers (motor) rather than indirect effects mediated by the afferent fibers (sensory) have not been clearly understood. Thus, we tested our hypothesis that VNS exerts cardioprotection predominantly through its efferent fibers. Thirty swine (25-30 kg) were randomized into 5 groups: I/R (no VNS), VNS (left cervical VNS applied at the onset of ischemia), LtVNX (VNS+left vagus nerve transection), RtVNX (VNS+right vagus nerve transection) and atropine (atropine 1 mg/kg was injected 15 min before VNS). Ischemia was induced by left anterior descending coronary artery occlusion for 60 min, followed by 120 min of reperfusion. Infarct size, arrhythmia score, left ventricular (LV), and mitochondrial functions were assessed. VNS (both intact and LtVNX) significantly decreased the infarct size and arrhythmia score, improved left ventricular function and attenuated cardiac mitochondrial dysfunction. However, LtVNX produced more profound cardioprotection, particularly infarct size

reduction and decreased arrhythmia score, compared to RtVNX. These beneficial effects of VNS were abolished by atropine. In conclusion, our findings suggest that VNS provides cardioprotection against I/R injury predominantly through its efferent fibers.

Keywords: cardioprotection, efferent fiber, ischemia/reperfusion, mitochondria, vagus nerve stimulation

S27-3

Selective optogenetic stimulation of efferent fibres in the Vagus nerve of a large mammal

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Background: Autonomic dysfunction is known to contribute to the progression of many cardiovascular diseases. Electrical stimulation of the vagus nerve has been pursued as a treatment for the purpose of restoring the autonomic balance. However, this approach lacks selectivity both in the fibre types activated and target organs effected.
Aim: Our aim was to selectively stimulate vagal efferent fibres projecting from neurons in the dorsal motor nucleus of the vagus (DMV) using an optogenetic approach in a large animal model.
Methods: Using lentiviral vectors expressing the light-sensitive channel rhodopsin variant, oChIEF, expressed under the control of the PRSx8 promoter that selectively targets vagal preganglionic neurones, we targeted a subset of vagal motorneurons in the left DMV in 7 Merino ewes.
Results: Twelve weeks after the injection of the vectors, sheep expressed optogenetic channels in a subset of efferent vagal fibres at the level of the cervical vagus, cardiac vagus and reaching beyond the level of the diaphragm. Blue laser or LED light (>10 mW/mm^2; 1 ms pulses) applied to the cervical vagus triggered precisely timed, strong bursts of efferent activity with evoked action potentials propagating at speeds of ~6 m/s.
Conclusions: These findings show that in sheep, with large, multi-fascicled vagus nerves, it is possible to stimulate a specific sub-population of efferent fibres using light at a site remote from the vector delivery. This marks an important step forward for the use of optogenetic technology for autonomic neuromodulation.

Keywords: vagal nerve stimulation, optogenetics, heart failure, autonomic dysfunction

S27-4

Clinical application and vagus nerve stimulation

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The vagus nerve, a pivotal component of the autonomic nervous system, plays a multifaceted role in regulating various physiological functions. Vagus nerve stimulation (VNS) has emerged as a therapeutic modality with diverse clinical applications, revolutionizing the management of numerous medical conditions. This abstract provides a concise overview of the clinical applications of VNS, highlighting its therapeutic potential and the underlying mechanisms.
VNS is most notably utilized in the treatment of drug-resistant epilepsy, where it has demonstrated remarkable efficacy in reducing seizure frequency and severity. Furthermore, it has found applications in the management of treatment-resistant depression, with several studies reporting significant improvements in mood and quality of life in affected individuals. Recent research also suggests promising prospects in using VNS to address neuroinflammatory disorders such as rheumatoid arthritis and inflammatory bowel disease.
Beyond neurological and psychiatric conditions, VNS has shown promise in the realm of chronic pain management, particularly for patients with intractable pain syndromes. Its ability to modulate inflammatory responses and regulate the release of neurotransmitters makes it a versatile tool for pain control.
The mechanistic underpinnings of VNS involve its influence on the cholinergic anti-inflammatory pathway, which modulates immune responses and mitigates inflammation. Additionally, VNS affects various brain regions, including the limbic system and prefrontal cortex, contributing to its mood-enhancing and antidepressant effects.
In summary, vagus nerve stimulation has evolved as a groundbreaking therapeutic intervention with a broad spectrum of clinical applications. Its potential to ameliorate drug-resistant epilepsy, depression, chronic pain, and inflammatory disorders underscores its versatility and offers a promising avenue for improving the quality of life for individuals afflicted with these conditions. Further research is warranted to explore its full range of therapeutic possibilities and refine treatment protocols for optimal outcomes.

Keywords: vagus nerve stimulation, automonic nerve system, mental health, depression, HRV

S28-1

Neuroimmunity in pain: Role of natural killer cells

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Recently, role of neuroimmune interaction has been the subject of significant interest in pain research. We have demonstrated the resolution of persistent painful neuropathy through the clearance of partially damaged sensory nerves by innate immune natural killer (NK) cells. Based on this work, I will present sciatic partial crush injury (PCI) model as a new preclinical model which is suitable to study both peripheral nerve regeneration and pain in the spinal system, and also discuss potential therapeutic targets for NK cells which might be utilized for the treatment of chronic neuropathic pain. Further translational and clinical research, along with mechanistic studies in preclinical models, are required to address whether NK cell immunotherapy is a promising alternative to opioid drugs for the effective management of chronic neuropathic pain.

S28-2

Differential roles of anterior paraventricular nucleus in regulating mechanical hypersensitivity and aversion behavior in formalin pain model

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We showed previously that the anterior nucleus of paraventricular thalamus (PVA) plays an important role in chronic pain. Inhibiting PVA neuron activity alleviate mechanical hypersensitivity in different pain models. At the circuit level, PVA is connected to the central amygdala (CeA), contributing to the formation of mechanical pain. However, the cell type specificity and functional roles of PVA in different pain behaviors are still not clear. Anatomically, PVA is connected to regions associated with pain perception, indicating its importance in pain transmission pathways.
In this study, we demonstrated that intra-plantar injection of formalin activates PVA VGluT2+ neurons. Additionally, we identified PVA as a critical brain structure regulating mechanical hypersensitivity and aversion behavior in mice. Our data revealed that PVA VGluT2+ neurons project to the bed nucleus of the stria terminalis (BNST) and nucleus accumbens (NAc). Importantly, we found that the projections of PVA to BNST and NAc are anatomically and functionally segregated, highlighting their roles in modulating pain hypersensitivity and aversion behavior, respectively. The activation of BNST projections drives mechanical hypersensitivity, while activation of PVA projections to NAc induces aversion behavior. Indeed, our data suggest that PVA VGluT2+ neurons mediate mechanical hypersensitivity and aversion in the inflammatory pain model, and different projections mediate distinct aspects of pain behaviors.

Keywords: anterior nucleus of paraventricular thalamus (PVA), chronic pain, aversion behavior, mechanical hypersensitivity

S28-3

Functional and anatomical analysis of the connections between neurons activated in the process of pain chronification

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Pain is not simply a nociceptive sensation but rather a "warning signal" generated by the brain, which causes an “unpleasant sensory-emotional experience”. Our focus has been on the central amygdala (CeA), which receives non-thalamocortically mediated nociceptive/aversive information through the lateral parabrachial nucleus (LPB). The LPB-CeA system serves as a core component of the pain warning system and plays an essential role in the development of "nociplastic pain," a mechanism characterized by an altered nociceptive system. Using electrophysiological approaches, MRI network imaging, and behavioral analysis, we have been investigating the plasticity of the LPB-CeA synapse and demonstrated that the neural circuitry of the CeA undergoes plastic changes after transient inflammatory nociception, and that its activity, in turn, regulates pain behavior for a more extended period (Miyazawa et al., 2017; Arimura et al., 2019; Sugimoto et al., 2021; Yajima et al., 2022). To investigate the plasticity of the LPB-CeA synapse with the identification of involvement in nociceptive signaling during the pain chronification process, we utilized the FosTRAP2::Ai14 mouse line and Cre-dependent channelrhodopsin (ChR2) -YFP injected into the LPB. We investigated synaptic transmission between PB and CeA 'pain-activated neurons' identified by controlled Cre recombinase expression in a timing during the pain chronification process. Several weeks after orofacial formalin injection, followed by 4-hydroxytamoxifen administration at the 5-hour, we made brain slices and recorded LPB-CeA synaptic transmission elicited by light stimulation of ChR2 on LPB neuron terminals. We found that synaptic transmission between pain-activated LPB neurons and pain-activated CeA neurons was more robust than non-specific neuron combinations, particularly in the posterior CeA. These results suggest robust synaptic connectivity between neurons activated by nociceptive information.

Keywords: the central amygdala, The lateral parabrachial nucleus, FosTRAP, inflammatory pain, electrophysiology

S28-4

Personalized functional brain models of chronic pain

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The current assessment of chronic pain lacks objective biomarkers. Neuroimaging has demonstrated its potential as a surrogate marker of subjective pain in healthy populations, but its validity for individual patients with chronic pain remains uncertain. We conducted a functional Magnetic Resonance Imaging study involving patients with fibromyalgia, a condition characterized by chronic widespread pain. To obtain comprehensive and personalized models of individual patients, we extensively sampled each patient's brain activity patterns by scanning each patient for more than 20 sessions on different days. For each session, we conducted three runs of 10-minute fMRI scans while participants provided self-reports of spontaneous pain ratings. Using machine learning algorithms, we were able to identify personalized fMRI biomarkers capable of decoding the continuous fluctuations of spontaneous pain experienced by each participant.

Keywords: fMRI, biomarker, extensive sampling, neuroimaging, chronic pain

S52-1

Special Neuro-glia-vascular interactions in different brain regions uncovered through use of classical and advanced microscopy

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Approximately 140 years have passed since the discovery of the Blood-Brain Barrier (BBB) and 55 years since its cellular components were identified through tracer challenges and electron microscopy. Now, new imaging approaches are being developed to uncover unique brain barrier structures and special neuro-glia-vascular interactions in different brain regions.
Recent evidence suggests that there is molecular heterogeneity among the different components of the Neurovascular Unit (NVU), particularly among endothelial cells, across different types of vessels in the Central Nervous System (CNS). These findings, mainly obtained from single-cell mRNA sequencing profiling, have led us to investigate the possibility of

cell biological and functional heterogeneity in barrier properties.
In this talk, I will showcase the utilization of new imaging techniques, including Stochastic Optical Reconstruction Microscopy (STORM) for super-resolution imaging, and serial electron microscopy image analysis. I will demonstrate the use of these new imaging approaches in combination with tracer challenges and established imaging techniques such as confocal microscopy, and transmitted/scanning electron microscopy. By combining multiple imaging tools, we are able to discover atypical barrier structures. We focus on uncovering unique features of the arterial BBB, testing the boundaries of circumventricular organs (CVOs), and describing specialized neurovascular unit structures in the hippocampus.
Based on our imaging findings, we suggest shifting from a unifying view of the classical BBB to acknowledge structural and functional heterogeneity. Moreover, mounting evidence in our field is pointing at a notion that even the classical BBB structure might present dynamic functional changes that correspond with neuronal activity, disease state, circadian clocks, aging, and more. We anticipate that these findings are only the tip of the iceberg and that new imaging techniques will lead to numerous additional discoveries.

Keywords: BBB, neruovascular, super resolution

S52-2

Distinct function of common guidance cues in the nervous versus vascular system

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The nervous and vascular systems are both exquisitely sophisticated systems, and their proper establishment requires specific guidance of nerves and vessels during the remodeling process after pathological insults as well as during development. Accumulating studies reveal that the traditional axon guidance cues, mostly composed of ligand-receptor pairs, play a variety of roles in the establishment of structural and functional nervous and vascular systems. Interestingly, the common cues share many features in terms of molecular mechanism, but still possess a different tool in each system to achieve a unique function. In this talk, I will show the novel mechanism of Semaphorin 3E (Sema3E)-Plexin-D1 repulsive axon guidance cue during neuronal pathfinding. Although axon guidance molecules are critical for the extension of neuronal projections to target sites according to the designed program, it remains unclear how such cues coordinate axonal growth and turning. Recently, we found that Sema3E-Plexin-D1 signaling induces a specific pool of gene expression in the projecting striatonigral neurons, of which Mtss1, a membrane protrusion protein, plays a dual function, axonal projection and potentiation of repulsive guidance. Mtss1 can bind to Plexin-D1 and enables its localization to the growth cone, where it signals a repulsive cue to Sema3E. Thus, Mtss1 knockout mice exhibited fewer striatonigral projections and irregular axonal routes, and these defects were recapitulated in Plxnd1 knockout mice. These results demonstrate that repulsive axon guidance cues induced a dual-functioning facilitator through which navigating axons ensure incessant extension to their target tissues while showing sensitivity and subsequent steering in response to repulsive signals.

Keywords: axon guidance, neurovascular system, Semaphorin 3E, Plexin-D1, Mtss1

S52-3

Selective reorganization of excitatory synapses by astrocytic phagocytosis in the adult striatum

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Synapse turnover occurs dynamically throughout the animal's life and is important for regulating cognitive functions such as learning and memory. Our previous study showed that astrocytic synapse phagocytosis is required for the reorganization of excitatory synapses in the adult hippocampus and is essential for normal excitatory synapse homeostasis and memory formation. These findings raise the possibility that astrocytic phagocytosis mechanisms are also implicated in maintaining normal excitatory synaptic connectivity and related cognitive functions in other areas in the adult brain. Because the striatum is an important brain area regulating procedural and skill learning and memory, we examined whether astrocytic phagocytosis plays a role in striatal excitatory synapse turnover. Our results showed that excessive excitatory synapses are produced in the striatum with astrocytic knock-out of multiple epidermal growth factor-like domains protein 10 (*Megf10*) and MER proto-oncogene, tyrosine kinase (*Mertk*) genes ($Megf10^{-/-}$; $Mertk^{-/-}$). Remarkably, among multiple excitatory projections innervating the striatum, astrocytes prefer to target the corticostriatal pathway rather than thalamostriatal one, because the dorsal striatum with astrocytic $Megf10^{-/-}$; $Mertk^{-/-}$ contains excessive cortex-derived synapses but normal thalamus-derived ones. In line with these findings, we found that only corticostrital synaptic transmission was altered by astrocytic $Megf10^{-/-}$; $Mertk^{-/-}$ when the cortico- or thalamostriatal transmission was selectively evoked by optogenetic stimulation. Together, these results not only provide evidence for astrocytic reorganization of excitatory synapses in multiple brain areas, but also suggest the selective phagocytosis of corticostriatal synapses by striatal astrocytes.

Keywords: astrocyte, striatum, phagocytosis, synapse, corticostriatal projection

S52-4

Astrocytes in the lateral septum modulate stress responses

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Our daily life is exposed to diverse physiological and psychological stressors. The lateral septum (LS) is a critical relay station that integrates sensory and contextual information about stressors and promotes adaptive responses. It is the main downstream of the hippocampus and receives direct synaptic inputs from other cortical and subcortical regions. LS consists mainly of inhibitory neurons that project to many subcortical regions. They also send collateral projections to other neurons in the LS, which may produce a reciprocal lateral inhibition between LS neurons. It has been reported that LS neurons are activated by stressors, including startling visual and auditory stimuli, approaching predators, electric shock, and tail suspension stress. Functional studies have shown that the LS participates in diverse cognitive and emotional processes, including anxiety, fear, feeding, and social behavior, although the behavioral outcomes were often inconsistent between studies. Recently, the LS has been dissected into molecularly distinct neural subpopulations using molecular genetic tools, and the functional

heterogeneity of the LS has begun to be revealed.
The LS contains glial cells, such as astrocytes. It has been considered for a long that the main functions of astrocytes are restricted to providing homeostatic support for neurons. However, growing evidence indicates that they interact actively with local neural circuits and are critical in generating physiological and behavioral responses. Since the role of astrocytes in the LS has yet to be elucidated, my laboratory has used a combinatorial approach, including *in vivo* calcium recording, slice electrophysiology, optogenetic and chemogenetic manipulations, and behavioral analysis, to study the function of astrocytes in the LS. We have found that astrocytes in the LS exhibit calcium transients in response to diverse stressors. Chemogenetic activation of the Gq pathway in LS astrocytes elicited neural excitation in the LS and promoted anxiety-like behavior and social avoidance in stressed animals. These results suggest that astrocytes can modulate neural circuits for stress responses.

Keywords: lateral septum, astrocyte, stress, neural circuit, behavior

S30-1

Visualization and detection of the cellular energy currency with genetically encoded fluorescent biosensors

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Background: ATP is a primary cellular energy currency and plays a crucial role in various cellular processes. Therefore, real-time monitoring of ATP levels in living cells is essential for understanding cellular functions. In this study, we developed novel live-cell ATP imaging/detecting technology based on fluorescent proteins and applied it to visualize the ATP dynamics in various cell types.
Methods: We have developed two types of genetically encoded fluorescent biosensors for ATP, ATeam and QUEEN. A vital component of both biosensors is a bacterial FoF1-ε protein, which undergoes a large conformational change upon binding of ATP. ATeam, in which the FoF1-ε is sandwiched by a donor fluorescent protein and an acceptor fluorescent protein, is operated by the principle of Förster resonance energy transfer (FRET): ATP binding to FoF1-ε alters the distance between a donor and an acceptor, resulting in ATP concentration-dependent change in FRET efficiency. QUEEN, in which a circular-permuted GFP is inserted within the FoF1-ε, changes the fluorescence excitation spectrum in an ATP concentration-dependent manner. The cells introduced with the cDNA for these ATP biosensors were imaged with a fluorescent microscope or analyzed with a fluorescence-activated cell sorter.
Results: The developed method allowed us to visualize spatiotemporal dynamics in ATP concentration in various living cells, including cardiomyocytes, hepatocytes, pancreatic β-cells, cancer cells, and bacterial cells. Using this technique, dynamics, distribution, and diversity of ATP in various biological processes, including hypoxic response, viral infection, insulin secretion, cell division, and apoptosis, have been revealed.
Conclusion: Live-cell ATP imaging technology enabled real-time monitoring of ATP levels in living cells. This technique has broad applications in understanding the role of energy metabolism in biological phenomena at high spatial and temporal resolution.

Keywords: fluorescent protein, FRET, live imaging, ATP, bioenergetics

S30-2

Anti-aging gene Klotho in calcium signaling

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Klotho is a type I transmembrane anti-aging protein, that undergoes shedding of its extracellular domain, which is subsequently secreted into the systemic circulation. Both membranous and soluble forms of Klotho play pivotal roles in protecting multiple organs. Importantly, the Klotho gene has been associated with an increased risk for age-related diseases, and its deficiency in mice leads to accelerated aging and imbalance of ion homeostasis. Klotho's significance lies in its role as a major regulator of Ca^{2+}/Pi metabolism, the disturbance of which has been potentially linked to organ senescence. Emerging research has indicated that Klotho can mitigate organ senescence. Moreover, Klotho plays a critical role in regulating multiple Ca^{2+}-permeable channels, thereby stabilizing Ca^{2+} balance and suppressing overactivation of Ca^{2+} signaling-mediated pathological events. In this presentation, I will provide a concise overview of the current state of research on the impact of Klotho on Ca^{2+} signaling and organ protection aimed to elucidate potential mechanisms and highlight future directions in the field.

Keywords: Klotho, calcium, aging, channels, mineral metabolism

S30-3

Cereblon, a novel cardiac contractile regulator

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AIMS: Cereblon (CRBN) is a substrate receptor of the E3 ubiquitin ligase complex that was reported to target ion channel proteins. L-type voltage-dependent Ca^{2+} channel (LTCC) density and dysfunction is a critical player in heart failure with reduced ejection fraction (HFrEF). However, the underlying cellular mechanisms by which CRBN regulates LTCC subtype Cav1.2alpha during cardiac dysfunction remain unclear. Here, we explored the role of CRBN in HFrEF by investigating the direct regulatory role of CRBN in Cav1.2alpha activity and examining how it can serve as a target to address myocardial dysfunction.
METHODS AND RESULTS: Cardiac tissues from HFrEF patients exhibited increased levels of CRBN compared with controls. In vivo and ex vivo studies demonstrated that whole-body CRBN knockout (CRBN-/-) and cardiac-specific knockout mice (Crbnfl/fl/Myh6Cre+) exhibited enhanced cardiac contractility with increased LTCC current (ICaL) compared with their respective controls, which was modulated by the direct interaction of CRBN with Cav1.2alpha. Mechanistically, the Lon domain of CRBN directly interacted with the N-terminal of Cav1.2alpha. Increasing CRBN levels enhanced the ubiquitination and proteasomal degradation of Cav1.2alpha and decreased ICaL. In contrast, genetic or pharmacological depletion of CRBN via TD-165, a novel PROTAC-based CRBN degrader, increased surface expression of Cav1.2alpha and enhanced ICaL. Low CRBN levels protected the heart against cardiomyopathy in vivo.

Keywords: Cereblon, HFrEF, Cav 1.2alpha, contraction

S30-4

Redox-dependent alternative internalization (REDAI) of purinergic $P2Y_6$ receptor regulates colitis but not non-alcoholic steatohepatitis.

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G protein-coupled receptors (GPCRs) play pivotal roles in converting physicochemical stimuli due to environmental changes to intracellular responses. After ligand stimulation, many GPCRs are desensitized and then recycled or degraded through b-arrestin-dependent internalization, an important process to maintain protein quality control of GPCRs. However, it is unknown how GPCRs with low β-arrestin sensitivity are controlled. Here we unmasked a b-arrestin-independent GPCR internalization, named Redox-dependent alternative internalization (REDAI), using b-arrestin-resistant purinergic $P2Y_6$ receptor ($P2Y_6R$). Natural isothiocyanates (ITCs) covalently bind with Cys^{220} in the intracellular 3rd loop of $P2Y_6R$, and promote internalization and degradation of $P2Y_6R$ through ubiquitination of Lys^{137}in the 2nd loop. $P2Y_6R$ is highly expressed in macrophage and pathologically contributes to the development of colitis in mice. Endogenous electrophiles, such as S-nitrosoglutathione, also induce $P2Y_6R$ degradation leading to anti-inflammation in macrophages. Prevention of Cys^{220} modification on $P2Y_6R$ resulted in aggravation of the colitis. On the other hands, knockout of $P2Y_6R$ fails to improve liver injury and inflammation in non-alcoholic steatohepatitis. Accordingly, targeting REDAI on GPCRs will be a breakthrough strategy for the prevention and treatment of colitis.

Keywords: GPCR, $P2Y_6R$, NASH

S30-5

System analysis for alleviating muscle atrophy using medicinal herbs

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Muscle atrophy is a progressive loss of mass and function in skeletal muscle, characterized by an imbalance in the synthesis and breakdown of myofibrillar proteins. Muscle atrophy can be caused by a variety of factors, including natural aging (sarcopenia), and the number of people with sarcopenia is increasing rapidly, especially in an aging society. Muscle atrophy has a detrimental effect on quality of life, morbidity from various diseases, and mortality; however, effective and safe therapies to treat muscle atrophy have not yet been developed. To develop a strategy to alleviate muscle atrophy, we conducted a systematic transcriptome-based analysis to investigate the effects of the following herbs and ingredients: Jakyak-gamcho-tang (JGT), a decoction of Paeoniae Radix and Glycyrrhizae Radix et Rhizoma, and Ginsenoside Rc (gRc), a major component of *Panax ginseng*.
1) JGT has long been used to relieve muscle tension and control muscle cramp-related pain, but the effects of JGT on muscle atrophy are yet to be comprehensively clarified. We have demonstrated the protective effect of JGT in dexamethason(DEX)-induced muscle atrophy models and elucidate its underlying mechanism through integrated *in silico* – *in vitro* – *in vivo* studies. Preliminary transcriptome analysis revealed that JGT regulated various pathways related to muscle differentiation and regeneration. DEX-treated C2C12 myotubes and muscle tissues of atrophic mice displayed substantial muscle protein degradation and muscle loss, respectively, which was efficiently alleviated by JGT treatment. JGT-mediated protective effects were associated with preservation of mitochondrial function, upregulation of myogenic signaling pathways, including Akt/mTOR/FOXO3, inhibition of ubiquitin-mediated muscle protein breakdown, and downregulation of inflammatory and apoptotic pathways induced by DEX.
2) gRc has various pharmacological activities, but its effect on muscle loss remains poorly explored. We demonstrated the effectiveness of gRc on muscle atrophy due to oxidative stress induced by hydrogen peroxide (H_2O_2). gRc effectively suppressed cytotoxicity, intracellular reactive oxygen species (ROS), and mitochondrial superoxide production, restored PGC-1α promoter activity, and increased ATP synthesis in C2C12 cells. Moreover, gRc significantly affected the expression levels of genes involved in maintaining mitochondrial mass and biogenesis, while downregulating genes associated with muscle degradation in C2C12 myotubes under oxidative stress.
We provided compelling evidence supporting the potential of JGT and gRc as a promising treatment for muscle loss and weakness. Transcriptomic pathway analysis can be employed as an efficient *in silico* tool to predict novel pharmacological candidates and elucidate molecular mechanisms underlying the effects of phytochemicals as well as herbal medications comprising diverse biologically active ingredients.

Keywords: transcriptome, herbal medicine, natural product, network pharmacology, docking simulation

S37-1

Altered synaptic architecture in the human dysplastic neocortex

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Focal cortical dysplasia (FCD) is a developmental disorder of the cerebral cortex and a main cause of drug-resistant epilepsy in children. A loss of the excitation and inhibition (E/I) balance has emerged as a key pathological feature for neuronal hyperexcitability, underlying the pathogenesis of seizures. However, it remains unclear how synaptic ultrastructure is altered in the human patients with FCD. Here we performed morphological analyses on the density of both excitatory and inhibitory synapses as well as the distribution of presynaptic mitochondria and vesicles in the temporal cortical layer III of a patient with FCD using 3D electron microscopy. Our quantitation showed a reduced density of symmetric (inhibitory) synapses in the soma, but not in the dendrites, of pyramidal neurons. Notably, we also found a reduction in asymmetric (excitatory) synapses in the dysplastic region compared to control. Further analyses of excitatory synapses revealed that the volume of dendritic spines and the size of synapses were markedly enlarged in the dysplastic region, with a concomitant increase in the number of synaptic vesicles. Moreover, the distance from inhibitory synapses to the nearest excitatory synapses along the dendrite was significantly lengthened in the dysplastic region, which would compromise effective inhibition over excitation. Additionally, the densities of both presynaptic boutons containing mitochondria and postsynaptic protrusions harboring a spine apparatus (SA) were markedly decreased in the epileptogenic region, suggesting defects in intracellular calcium buffering and synaptic plasticity. Finally, maladaptive myelination was prominent in the dysplastic region. Collectively, these disturbances of synaptic structures may represent

pathogenetic mechanisms underlying the neuronal hyperexcitability of the human patient with FCD.

Keywords: cortical dysplasia, epilepsy, synapse, mitochondria, electron microscopy

S37-2

Climbing fiber to interneuron synapse supports cerebellar motor learning

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The cerebellum codes for modulatory signals for motion and cognition. The importance of inhibitory interneurons (IN) in the cerebellar computation has been recognized recently. To further understand the roles of the INs, we reconstructed and analyzed the neural circuits in serial block-face electron microscope images using a new synapse detection method. We redefined the types of INs according to their input and output specificity, which form a disinhibition pathway with other types: climbing fiber (CF) - IN2 - IN1 - Purkinje Cell (PC). The connectivity-based type is independent of the basket vs. stellate types. In the conventional connectivity and learning model, simultaneous parallel fiber (PF) and CF input should weaken PF to PC synapses by long-term depression (LTD). However, the IN inputs to PC which are necessary for cerebellar computation may prevent the LTD. The CF-triggered disinhibition can ensure the LTD by silencing the IN input to PC. These findings agree with molecular and physiological studies.

Keywords: cerebellum, motor learning, disinhibition, interneuron, climbing fiber

S37-3

Presynaptic regulation of dendritic segment-selective synaptic plasticity in motor learning

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The architecture of neural circuits that underlies a variety of brain functions involves spatiotemporally-selective synaptic connections among neurons. Although the wiring diagram, "connectome", composed of diverse cell types in the neocortex has been described anatomically, this neural circuit map lacks the information about the role that each wiring plays in a particular function. To unveil the function of neural circuits involved in animal's behavior in vivo, I focused on synaptic remodeling in motor skill learning. Learning novel motor skills rewires neuronal connections by generating new synapses in the motor cortex. Of particular importance are the spines on pyramidal cell dendrites where synapses are potentiated, formed and maintained during learning. Since both corticocortical and thalamocortical fibers converge on the apical tufts of pyramidal cell dendrites, we characterized the presynaptic axon terminals innervating newly-formed spines. We observed spine dynamics under a two-photon microscope while Thy1-eGFP-M mice learned a forelimb motor task, and subsequently fixed the brains to prepare thin sections for immunohistochemistry. Post hoc characterization of the presynaptic axon terminals on new spines revealed that motor skill improvement coincided with selective formation of spines innervated by corticocortical axons. In addition, electron microscopy showed that the presynaptic axons made relatively small contacts with the postsynaptic spines formed during learning, suggesting that long-term contribution to the acquired motor skill requires the enlargement and maturation of the new synapses. The quantification of spine-head sizes revealed structural long-term potentiation of thalamocortical synapses, indicating that thalamocortical synapses were generated fewer during motor learning but survived longer and increased spine size more than new corticocortical synapses. The input-dependent reconfiguration of learning-related new spines suggests that transient corticocortical synaptogenesis contributes to skill improvement while new thalamocortical connections are sustained for retention of the acquired motor memory. Thus, observation of synaptic dynamics based on the subcellular "connectome" may allow the description of "functional connectome" that underlies multimodal information processing in the neocortex.

Keywords: neocortex, learning, synapse, plasticity

S37-4

A large volume EM data set and microcircuit analysis of neocortex

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Analysis of synaptic connections between neurons in a volume EM is a powerful research tool to investigate architecture of neural microcircuit in brain. The techniques have been improved quite significantly during the past two decades. It includes focused ion beam (FIB)-scanning electron microscopy (SEM), serial block face (SBF)-SEM, automated tape collecting ultramicrotome (ATUM)-SEM, and ATUM-transmission electron microscopy (TEM). I would like to introduce outline and the latest advances of the ATUM-SEM and ATUM-TEM in my own laboratory. One of the distinguishing features of these approaches is the capability of large volume EM imaging. This state-of-the-art technology enables the image acquisition of brain tissue up to cubic millimeter or even larger volume. The volume can covers entire layer structure of rodent/monkey cortex and allows us to investigate wiring of microcircuit in a cortical column by 3D reconstruction of neural elements with artificial intelligence (AI)-assisted automated segmentation.
Using the large volume EM data set obtained with the ATUM-SEM, we investigated target structure of thalamo-cortical axon terminals in rat frontal cortex. A viral vector (pal-GFP AAV) was injected into motor-related thalamic nuclei: the ventral medial nucleus (VM), the ventral anterior (VA) and the ventral lateral (VL) thalamic complex, which relay motor information from the basal ganglia (VM/VA) and the cerebellum (VL), respectively. We developed a correlated light and electron microscopy (CLEM) with a laser confocal microscopy and ATUM-SEM to analyze the target structure. To identify the GFP labeled axonal fibers subsequently at the electron microscopy, we stained blood vessels with fluor-lectin, and cellular nuclei with DAPI, and used them as landmarks in cortical tissue sections. Firstly, images were taken with a laser confocal microscopy. Then the tissue sections were embedded in plastic and sectioned with ATUM for SEM observation. GFP-labeled thalamo-cortical fibers and their target structures were identified in serial electron micrographs, and reconstructed three-dimensionally. Our preliminary results indicated that the VA fibers mainly targeted dendritic spines of the layer 5 pyramidal cell.

Keywords: electron microscopy, large data, connectome, cortex, thalamo-cortical

S31-1

Mutations in RyR2 calcium binding residues of hiPSC-CMs disable CICR and reveal cardiac EC-coupling remodeling

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Cardiac EC-coupling is regulated by Calcium entry through the L-type Ca^{2+} channels that triggers Ca^{2+} release from the ryanodine receptor (RyR2). While this process is well studied little is known as to the molecular determinants of RyR2 Ca^{2+} binding residues that sense the influx of Ca^{2+} and the pathophysiological consequences of mutating these residues on cardiac function. Using CRISPR/Cas9 gene editing in human induced pluripotent stem cell (hiPSC) platform, we studied the effects of mutating 2 of the 5 residues in RyR2 Ca^{2+} binding pocket to examine the consequences of disabling CICR in human cardiomyocytes (hiPSC-CMs).

Methods: Ca^{2+}-signaling phenotypes of mutations in RyR2 Ca^{2+} binding site residues associated with cardiac pathology (RyR2-Q3925E and RyR2-E3848A) were determined using I_{Ca}- and caffeine-triggered Ca^{2+} releases in voltage-clamped and TIRF-imaged wild type (WT) and mutant cardiomyocytes infected with SR-targeted ER-GCaMP6 probe.

Results: In both mutant lines, I_{Ca-} and caffeine-triggered Fura-2 and ER-GCaMP6 signals were suppressed, while Ca^{2+} transported by I_{Ca} was greatly enhanced. Spontaneous beating (Fura-2 Ca^{2+}-transients) persisted, however, in mutant cells without significant SR Ca^{2+} -release signals. While 5-20mM Caffeine also failed to trigger Ca^{2+}-release in voltage-clamped mutant-cells, in intact mutant cells ~70% of myocytes responded to 20mM caffeine, but such Ca-transients activated slowly, were delayed, and variably suppressed by 2-APB, FCCP, or ruthenium red.

Conclusion: mutation of RyR2 Ca^{2+} binding residues suppress significantly I_{Ca}- and caffeine-triggered Ca^{2+} releases, suggesting knockdown of CICR without suppression of spontaneous and rhythmic release of calcium and beating. It is likely that the enhanced transmembrane Ca^{2+} influx and activation of other cellular Ca^{2+} pools compensate for the suppressed CICR suggesting significant remodeling of EC-coupling even in cell culture media that underlie their functional survival.

Keywords: cardiac ryanodine receptor, RyR2, calcium binding residues mutations, CRISPR/Cas9 gene edit, hiPSC-CMs, CICR

S31-2

How does calcium leak into cardiac myocytes? Consequences for arrhythmias

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The calcium concentration in a ventricular myocyte is about 10,000 times less than that of plasma and increases roughly tenfold during systole. The L-type Ca current is a well-characterized mechanism by which Ca^{2+} ions enter the cell and contribute to both the action potential and the generation of contraction. However, there is evidence that other mechanisms can contribute to Ca^{2+} influx but their identify and quantitative importance is unknown (PMID: 16563501; PMID: 31999537). The aim of this work was to characterize this "background" Ca^{2+} influx.

Experiments were performed on sheep ventricular myocytes. Ca^{2+} entry was increased by elevating external Ca^{2+} concentration. This resulted in the occurrence of waves of Ca^{2+} release from the sarcoplasmic reticulum (SR) indicating an increased SR Ca^{2+} content. The Ca^{2+} waves activate sodium-calcium exchange and the resulting depolarizing current has been shown to produce arrhythmogenic afterdepolarizations. This current also gives a measure of the amount of Ca^{2+} pumped out of the cell, from which Ca^{2+} influx is measured. We found that the influx was inhibited by a TRPC6 inhibitor (BI 749327). This inhibitor also decreased Mn^{2+} influx into the cell, a measure of Ca^{2+} influx, again suggesting a role for TRPC6 in background Ca^{2+} entry (PMID: 35233776). We also found that the background influx is increased in myocytes from sheep with heart failure (rapid pacing). This extra Ca^{2+} influx may therefore contribute to the arrhythmias observed in heart failure. Finally, we find that the phosphodiesterase 5 inhibitor sildenafil decreases the background influx and this is associated with a decrease of arrhythmias (PMID: 34247494). Further work is required to clarify the role of this background Ca^{2+} entry in normal cardiac physiology.

Keywords: calcium, arrhythmia

S31-3

Exercise-induced cardiac remodeling

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The heart is the primary pump that circulates blood through the entire cardiovascular system, serving many important functions in the body. Exercise training provides favorable anatomical and physiological changes that reduce the risk of heart disease and failure. Compared with pathological cardiac hypertrophy, exercise-induced physiological cardiac hypertrophy leads to an improvement in heart function. Exercise-induced cardiac remodeling is associated with gene regulatory mechanisms and cellular signaling pathways underlying cellular, molecular, and metabolic adaptations. We found that aerobic exercise training decreased cereblon (CRBN), a substrate recognition protein in the E3-ligase ubiquitin complex. The binding target of CRBN varies according to tissues and cells, and the protein regulates various biological functions by regulating tissue-specific targets. As new endogenous targets of CRBN have been identified over the past decade, the physiological and pathological functions of CRBN and its potential as a therapeutic target in various diseases have greatly expanded. Here, I will present a cellular and molecular signaling pathway of CRBN to understand the exercise-induced cardiac adaptation.

Keywords: exercise, cereblon, cardiac adaptation

S31-4

Distinct alterations in local calcium signaling in right and left atrial myocytes in a rat model of pressure overload

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Ca^{2+} releases in the interior of atrial myocytes, lacking transverse tubules, govern their contractility, but their regulatory mechanisms are poorly understood. Loss of atrial contractility is associated with atrial blood stasis and decrease of ventricular filling at diastole. The conditions such as atrial fibrillation (AF) can be caused by high hemodynamic disturbance including shear stress and pressure overload. AF indeed

occurs more often in left atrial (LA)- than right atrial (RA)-chamber. We investigated if and how Ca^{2+} releases from the cell interior versus peripheral junctional Ca^{2+} release are altered by prolonged increase in afterload and by high fluid shear stress, and compared these responses between LA and RA myocytes. Rapid 2-D confocal Ca^{2+} imaging was used to simultaneously measure peripheral and non-junctional (central) Ca^{2+} releases in rat atrial myocytes. Transverse aortic constriction (TAC) for >20 weeks was used to induce left heart failure in rats, and shear stress was applied onto single cell by pressurized fluid-puffing. Shear stress transiently enhanced peripheral and central Ca^{2+} releases on depolarization with much higher stimulation on non-junctional sites, which was followed by attenuations of Ca^{2+} transients at both sites. At high rate of depolarizations, this shear-induced large central Ca^{2+} increase was removed only in LA cells, where fractional release was also significantly lower than RA myocytes. After 20-week TAC, Ca^{2+} transients were reduced in LA cells but enhanced in RA cells with similar decrease in their SR Ca^{2+} loading. In addition, central release efficacy at a given peripheral release on depolarization was increased in both RA and LA cells. Peripheral Ca^{2+} release was reduced more in LA cells from TAC rats, while central (non-junctional) Ca^{2+} release was enhanced in TAC RA cells. Decay and rise of Ca^{2+} signals were somewhat accelerated in TAC RA cells, whereas they were slowed in TAC LA cells. Immunoblotting analyses revealed that RA and LA cells from TAC rats have differential profile of Ca^{2+} signaling toolkit gene expressions, in particular, for ryanodine receptor, L-type Ca^{2+} channel, and phospholamban, which was consistent with distinct alterations in local Ca^{2+} signaling on depolarizations between RA and LA cells from failed hearts. Our data provide new evidence on distinct deterioration in non-junctional Ca^{2+} releases in LA myocytes, but not in RA cells, under high beating rate and shear stress as well as prolonged pressure overload. This may give an insight on higher incidence of AF in left atrium.

Keywords: atrial Ca^{2+} signaling, transverse aortic constriction, heart failure, left atrial, right atrial

S31-5

Splicing variants of neuronal nitric oxide synthase in the heart - Ca regulation and beyond

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nNOS gene generates 5 alternative splicing variants (nNOS-α, nNOS-β, nNOS-μ, nNOS-γ, and nNOS-2), among which nNOS-α, nNOS-β, nNOS-μ show the property of transferring electrons from oxygenase to reductase domain, therefore contributes to NO bio-availability in the cells. Spatial confinement of nNOS splicing variants defines diverse mode of the regulations. E.g. nNOS-α and nNOS-μ interact with target proteins in N-terminal *via* the PDZ binding motif, are spatially confined in the plasmalemma and cytosol, modulates ion channel activities, intracellular Ca transients, contraction and redox regulations. nNOS-β, on the other hand, is expressed in the myofilament fraction of cardiomyocytes. Here, I will discuss our recent findings of nNOS-β in cardiomyocytes. In particular, functional roles of nNOS-β protein in sarcomere structure and dynamics in both healthy and angiotensin II-induced hypertensive rat hearts. The study provides a new conceptual framework of nNOS splicing variants in preventing the patho-progression of cardiac hypertrophy under pressure-overload.

Keywords: nNOS-beta, Heart, Ca regulation, Sarcomere dynamics, Hypertrophy

S32-1

The role of calpain protease and vimentin cleavage in the signal transduction of abnormal vascular smooth muscle contraction

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Rho-kinase (ROK) modulates the phosphorylation level of myosin light chain (MLC) and plays a critical role in the signal transduction of abnormal vascular smooth muscle (VSM) contraction leading to vasospasm. Previously we identified sphingosylphosphorylcholine (SPC)/Fyn/ROK pathway which mediates abnormal VSM contraction. As possible downstream targets of Fyn tyrosine kinase, we identified vimentin by focused proteomics. Interestingly, SPC induced limited proteolysis of vimentin in human coronary artery smooth muscle cells (CASMCs) and VSM strips of the porcine coronary artery. Since vimentin is reported as the target of calpain, we examined the involvement of calpain. In CASMCs, SPC increased calpain activity, which was blocked by PD150606, a calpain inhibitor. Furthermore, PD150606 inhibited the SPC-induced VSM contractions, ROK activation and MLC phosphorylation, suggesting that calpain is involved in the signal transduction of abnormal VSM contraction mediated by the SPC/Fyn/ROK pathway. To clarify the role of vimentin cleavage in the signal transduction of abnormal VSM contraction, we overexpressed full-length vimentin and vimentin fragments (N-terminal and C-terminal) whose length were corresponding to the vimentin fragments generated by the limited proteolysis in CASMCs. Whereas the full-length vimentin exhibited filament assembly as well as endogenous vimentin did, either N-terminal or C-terminal vimentin fragment demonstrated impaired filament assembly. Interestingly, we observed the overexpression of N-terminal vimentin fragment, but not of C-terminal vimentin fragment, induced caspase-negative cell rounding. Those findings suggested the N-terminal vimentin fragment might facilitate the signal transduction of abnormal VSM contraction.

Keywords: abnormal vascular smooth muscle contraction, calpain, vimentin

S32-2

A role for HDAC1/c-Myc axis in obese hypertension

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High-fat diet (HFD)-induced obesity is a cause of resistant hypertension, whereas the underlying mechanisms remain to be elucidated. Here, we determined the role of HDAC1 in HFD-induced hypertension and found the pathologic axis between HDAC1 and renal angiotensinogen (Agt) expression. Treatment with HDAC1/2 selective inhibitor FK228 canceled the increased blood pressure of male C57BL/6 mice induced by HFD. FK228 also blocked the upregulation of renal Agt mRNA, protein, and angiotensin II (Ang II). Activation and nuclear accumulation of HDAC1 occurred in the HFD group. The activated HDAC1 deacetylated c-Myc transcription factor. HDAC1 knockdown increased c-Myc acetylation reducing its expression. Silencing of HDAC1 or c-Myc in HRPTEpi cells decreased Agt expression. Chromatin immunoprecipitation assay revealed that HFD induced the binding of HDAC1 and deacetylated c-Myc complex at the *Agt* gene promoter. A putative c-Myc binding sequence in the promotor region was necessary for Agt transcription.

Inhibition of c-Myc downregulated Agt and Ang II levels in the kidney, ameliorating HFD-induced hypertension. The results demonstrate the pathologic HDAC1/c-myc signaling axis in the kidney as a promising therapeutic target for obesity-mediated resistant hypertension.

Keywords: obesity-mediated hypertension, HDAC1, c-Myc, angiotensinogen, epigenetic regulation

S32-3

Unique responsiveness of pulmonary artery smooth muscle to thrombin as a promising target for treatment of pulmonary hypertension

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Background: Pulmonary hypertension (PH) is an intractable disease that is characterized by a progressive increase in pulmonary vascular resistance due to vasoconstriction, vascular remodeling and thrombosis. The development of specific drug therapy over the last 20 years has significantly improved the prognosis of the patients with PH; however, the patients in the advanced stage remain in poor prognosis. Better understanding of the physiology of pulmonary circulation and the pathophysiology of PH is required to further improve the outcome of PH treatment. PH is frequently associated with increased coagulability, formation of thrombosis and thrombotic pulmonary arteriopathy. Coagulation factors are known to exert vascular effects, which are related to vasoconstriction and vascular remodeling, by acting on a unique family of G protein-coupled receptor, referred to as proteinase-activated receptor (PAR). PAR is thus anticipated to be a novel therapeutic target for the treatment of PH.
Main observations: We found a unique property of the pulmonary artery that contracts in response to thrombin via PAR_1, while an endothelium-dependent relaxant response is the most frequently reported vascular effects of PAR_1 in the normal artery of the systemic circulation, and the contractile effect is reported in the limited arteries. The unique responsiveness of the pulmonary artery is supported by the finding that the level of mRNA expression of PAR_1, but not PAR_2-PAR_4, in the rat pulmonary artery is higher than that seen in several arteries of the systemic circulation. The level of PAR_1, but not PAR_2-PAR_4, was upregulated in the pulmonary artery of the monocrotaline (MCT)-induced PH model rats. The contractile response to PAR_1 agonist peptide was enhanced in MCT-PH rats, while no contractile response was observed for the agonists of PAR_2 or PAR_4. Treatment of MCT-PH rats with PAR_1 antagonist prevented the increase in right ventricle (RV) systolic pressure, RV hypertrophy and medial wall thickness of pulmonary artery, and prolonged the survival. Increase in RV systolic pressure and RV hypertrophy induced by chronic hypoxia in mice was mitigated in the PAR_1-knockout mice.
Conclusion: The pulmonary artery has a unique property regarding the PAR_1-mediated responsiveness to thrombin, which contracts pulmonary artery under physiological condition. The enhancement of PAR_1 expression and activity contributes to pathogenesis and pathophysiology of the experimental PH. PAR_1 has a potential as a novel therapeutic target for the treatment of PH.

Keywords: coagulation, proteinase-activated receptor, pulmonary hypertension, receptor antagonist

S32-4

Phenotypic modulation of vascular smooth muscle cells

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Since chronic inflammation are associated with the pathogenesis of atherosclerosis, inflammatory cytokines might contribute to the phenotypic modulation of vascular smooth muscle cells (VSMCs). TNFα facilitated the change of contractile VSMCs to the synthetic phenotype as determined by the expressions of marker proteins and collagen gel contraction assay results. Western blot analysis and promoter assay of cyclooxygenase-2 (COX2) revealed TNFα stimulation resulted in the induction of COX2. Overexpression, silencing, or pharmacological inhibition of COX2 significantly affected TNFα-induced phenotypic conversion, and of the tested prostaglandins, only PGD_2 significantly induced phenotypic conversion. ERK was significantly activated by PGD_2 stimulation and pharmacological inhibition of ERK blocked the PGD_2-induced phenotypic conversion of VSMCs. However, antagonists or agonists of PGD_2 receptors did not affect VSMCs conversion. By contrast, spontaneously dehydrated forms of PGD_2, such as, PGJ_2, Δ^{12}-PGJ_2, and 15-d-PGJ_2 strongly induced phenotypic conversion. A reporter gene assay showed that TNFα, PGD_2, and 15-d-PGJ_2 significantly activated peroxisome proliferator responsive element (PPRE) promoter. In addition, the overexpression or silencing of peroxisome proliferator-activated receptor δ (PPARδ) significantly influenced 15-d-PGJ_2-induced phenotypic conversion. Finally, atherosclerotic neointima formation was significantly suppressed in mice lacking TNFα. In addition, feeding mice celecoxib completely blocked carotid artery ligation-induced neointima formation. This study shows PGD_2 regulates the phenotypic conversion of VSMCs by generating an endogenous ligand for PPAR, and that this leads to neointima formation in a background of occlusive arterial disease.

Keywords: vascular smooth muscle, phenotype, PGD2, COX, inflammation

S32-5

Matrix-mediated mechanotransduction underlies vessel wall remodeling

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Extracellular Matrix (ECM) plays a role in activating intracellular signal transduction through the interaction between ECM and cells. In blood vessels, the response to mechanical signals such as blood flow and blood pressure accompanying hemodynamic stress plays a crucial role in maintaining vascular wall homeostasis and the onset of pathologies. However, how ECM functions during mechanical stress response and how the remodeling mechanisms of the vascular wall are interconnected to maintain vascular wall homeostasis in response to changes in the extracellular environment are not yet fully understood. In our research laboratory, we have identified Thrombospondin-1 (Thbs1), a matricellular protein, as an ECM molecule responsible for the mechanical stress response in the aortic aneurysm vascular wall (Yamashiro. Sci. Signal., 2015, Yamashiro. Circ. Res., 2018). Furthermore, we have revealed that Thbs1 secreted in response to stretching stimuli regulates the nuclear translocation of Yes-associated protein (YAP), a mechanosensitive transcriptional coactivator, through cell adhesion

complex integrin avß1 (Yamashiro. PNAS, 2020). In Thbs1-deficient mice, Thbs1/integrin avß1/YAP signaling is inhibited, leading to maladaptive remodeling of the aorta in response to pressure overload, resulting in aortic dissection, and it has been clarified that Thbs1 also participates in neointimal proliferation during carotid artery ligation. As recent findings, we have elucidated that neointimal cells causing vascular stenosis originate from endothelial cells (EC) and that partial Endothelial-to-Mesenchymal Transition (EndMT) is involved in its molecular mechanism (Yamashiro. Cardiovasc. Res., 2022). In this research conference, we aim to present the latest insights into the function of ECM in maintaining vascular wall homeostasis and the mechanism of EndMT, where endothelial cells undergo differentiation into smooth muscle-like cells. Additionally, we seek to deepen the discussion on how cells recognize their intra- and extra-cellular environments and regulate multicellular systems.

Keywords: ECM, mechanotransduction, EndMT, vascular biology

S33-1

Stress evokes mental disorder like behaviors via astrocytic MERTK-dependent synapse phagocytosis

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Childhood neglect/abuse can induce mental disorders with unknown mechanisms. Here, we identified stress hormones as strong inducers of astrocyte-mediated synapse phagocytosis. Our *in vitro*, *in vivo*, and brain organoid experiments showed that stress hormones significantly increased the astrocytic expression of the *Mertk* phagocytic receptor, through glucocorticoid receptor (GR). In postnatal mice, exposure to early social deprivation (ESD) resulted in specific activation of the GR/MERTK pathway in astrocytes but not in microglia. The excitatory postsynaptic density in cortical regions was significantly reduced in ESD mice with an increase in astrocytic engulfment of them. Remarkably, the loss of excitatory synapses, abnormal neuronal network activities, and behavioral abnormalities in ESD mice were substantially prevented by ablating GR or MERTK in astrocytes. Thus, our work reveals critical roles of astrocytic GR/MERTK activation in evoking stress-induced abnormal behaviors in mice, suggesting GR/MERTK signaling as a therapeutic target for the stress-induced mental disorders.

Keywords: astrocytes, microglia, synapse elimination, phagocytosis, mental disorder

S33-2

Probing behaviorally consequential astrocyte ensembles

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The control of behavior by neural circuits is much more complex than originally conceived by neuroanatomists in the late 19th century. Current understanding suggests that circuits are composed of a variety of cellular activities that are distributed throughout the brain, spinal cord, and peripheral regions, dynamically regulating the entire body system via sub- and supra-second signals. This complexity is due in part to the coexistence of multiple cell types within circuits. Astrocytes, which tile the entire central nervous system, play crucial roles in maintaining brain homeostasis. Recent studies using single-cell RNA sequencing have identified molecular diversity among astrocytes. However, it remains unclear whether functionally distinct astrocyte ensembles regulate specific behaviors. In this study, we aim to address this question using advantageous tools to quantify astrocyte anatomy, molecular properties, and causal impact in response to distinct valence-experiences. Our findings demonstrate that anatomically distinct astrocyte ensembles respond to different valence-stimuli and have unique molecular phenotypes. Whole brain mapping revealed distinct distributions of activated astrocytes in response to appetitive or aversive experience. Our data suggest that astrocytes responding to reward and aversion may be anatomically and molecularly distinct ensembles. We will also report our pilot experiments to elucidate the circuits and behavioral functions of these astrocytes.

Keywords: glia, astrocyte, behavior, circuit

S33-3

The experience of stress resulting from dietary factors or social interactions elicits glial reactivity in specific brain regions and the development of mood disorder

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Microglia (the CNS resident macrophages) and astrocytes (the major glial cell type) contribute to CNS homeostasis. Both glial cell types are also implicated in neuroinflammation associated with metabolic disorders. Stress associated with metabolic dysfunction or social impairment is a recognized risk factor for neurological and mood diseases. In our previous study, the intermittent peripheral immune challenges administered by inflammagen lipopolysaccharide (LPS) both before and after significant increases in body weight in HFD-fed mice can prime microglia in the hypothalamic ARC and other brain regions, rendering them more sensitive to the effects of chronic HFD feeding. This increased sensitivity may in turn counteract the impact of LPS on the development of abnormal exploratory behavior.

Altered dopamine (DA) transmission in the frontal striatum (FS) circuitry has been implicated in the development of depression. However, the specific effects of glial reactivity in the striatum on chronic stress related to obesity are still not well understood. Recent observations have revealed an increased presence of CD11b-immune cells in the meninges of male mice fed a high-fat diet (HFD), indicating a potential link between peripheral and meningeal inflammation. Immunofluorescence analysis demonstrated activated microglia and hypertrophic astrocytes in the caudate putamen (CPu) as a result of chronic HFD feeding. Interestingly, a decrease in aquaporin 4 (AQP4) levels, typically expressed in astrocyte endfeet, was detected in the CPu, along with reduced expression of the vascular marker CD31. Additionally, post-weaning isolation (PWI) disrupted the distribution of AQP4 and the vascular system in the CPu of female mice through D1R signaling. Moreover, PWI impaired the white matter structure in female mice exposed to HFD feeding. In conclusion, our findings indicate that chronic HFD feeding-induced stress could potentially contribute to gliosis and endothelial dysfunction in the CPu of adult male mice. Furthermore, disruption of AQP4-mediated water flux in the CPu and impairment of myelin in the corpus callosum of PWI-treated obese

female mice could potentially be restored through a D1R-associated signaling pathway.

Keywords: microglia, gliosis, neuroinflammation, myelin, aquaporin 4

S33-4

Divergent physiological functions of TDP-43 in distinct glia

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Common genetic loci and pathological signatures have unified two seemingly different adult-onset neurodegenerative diseases, amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), which affect predominantly the motor system and cognition, respectively. In particular, mutations in TDP-43 are causal for both diseases coupled with the pathological TDP-43 inclusions present in the neurons and glia indicate that TDP-43 dysfunctions in these cells trigger ALS and FTD pathogenesis. Furthermore, TDP-43 aggregates, collectively known as TDP-43 proteinopathies, are common in aging human brains and in other neurodegenerative diseases, such as Alzheimer's disease (AD), underscoring the critical role of TDP-43 in brain health.
TDP-43 is ubiquitously expressed. Curiously, pathological TDP-43 also can be found in neurons, glia and other peripheral systems. Two key questions: the physiological functions of TDP-43 in different cell types, and whether the loss of TDP-43 in distinct glia contribute to ALS/FTD pathogenesis, remain unresolved. To this end, we systematically analyzed mice with TDP-43 deleted in distinct glia, including oligodendrocytes, Schwann cells and astrocytes. We uncovered that (1) TDP-43 is indispensable for oligodendrocyte survival and myelination by regulating SREBF2-mediated cholesterol metabolism, (2) TDP-43 is required for maximize conduction velocity by maintaining paranodal assembly in Schwann cells, and (3) TDP-43 maintains the protective status of astrocytes. Loss of TDP-43 function in each of the distinct glia results in motor deficits without apparent damage to motor neurons. These results highlight that TDP-43 participate in different physiological role in distinct glia, and TDP-43 dysfunction in different glia may be an integral part of ALS pathogenesis.

Keywords: ALS, FTD, TDP-43, cholesterol, glia

S33-5

Astrocytes in chronic disease-associated mental disorders

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The brain functions highly rely on the blood supply, which brings nutrients and metabolites through the blood-brain interface to affect brain activity and homeostasis. Chronic diseases, such as chronic kidney disease (CKD) and diabetes, cause excessive accumulation of metabolites not only in the blood that are supposed to be excreted by the renal system, but also in the brain which results in mental disturbance at the chronic stage. Of note, CKD patients undergo hemodialysis still have protein-bound uremic toxins accumulation in the blood and subsequent into the brain via their transporters or damaged blood-brain barrier. Here, we reported that astrocytes are the major target of CKD-derived protein-bound uremic toxin indoxyl 3-sulfate, which is also a potent endogenous ligand of aryl hydrocarbon receptor (AhR), in the CKD-associated mental disorders. We find that anxiety and memory impairment in the CKD mouse model are both ameliorated in astrocyte-specific Ahr conditional knockout mice (astrocytic Ahr-cKO). Glutamate transporter 1, the major transporter for maintaining glutamate homeostasis in the brain, is downregulated in CKD mouse brain as well as long-term treated primary astrocytes and glia-neuron mix culture in an AhR-dependent manner. High resolution confocal imaging further revealed that CKD/chronic I3S treatment disrupted perineuronal astrocytic processes, increased synaptotoxicity and heightened neuronal activity in the anterior cingulate cortex, which was ameliorated in the astrocytic Ahr-cKO. Thus, this study suggests that astrocytes are the major target of CKD-induced brain disorders. Astrocytic AhR is a potential target for treating brain diseases involving accumulation of pathological AhR ligands.

Keywords: chronic kidney disease, mental disorders, astrocyte, aryl hydrocarbon receptor, glutamate transporter-1

S34-1

Targeting tumor microenvironment networks for developing novel therapeutic strategies

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Tumor microenvironment (TME) is composed not only of cancer cells but stromal components including tumor vessels, cancer associated fibroblasts (CAFs) and immune cells. TME components interact each other directly and/or indirectly via various cytokines to promote tumor progression and metastasis. Transforming growth factor -β (TGF-β), which is abundant in various types of cancer, has been implicated in tumor progression. TGF-β-induced epithelial-mesenchymal transition (EMT) confers epithelial cancer cells with migratory and invasive characteristics, which results in tumor metastasis. Furthermore, during TGF-β-induced endothelial-mesenchymal transition (EndoMT), endothelial cells lose barrier functions and acquire mesenchymal phenotypes, leading to the formation of CAFs. However, the relationship between TGF-β-driven EMT and EndoMT has not been fully elucidated. Cancer cell-derived extracellular vesicles (EVs) have been suggested to induce the destabilization of normal blood vessels at the metastatic sites. We found that the EVs that originated from oral cancer cells that underwent EMT by TGF-β induced EndoMT, and induced intercellular gap formation which led to the loss of endothelial cell barrier stability. Furthermore, we showed that TGF-β treated endothelial cells secrete TGF-β2, which induced EMT of oral cancer cells. Collectively, our findings suggest that TGF-β-driven EMT and EndoMT mutually interact via EVs and TGF-β2 which contributes to tumor progression. Targeting such TME network signals is expected to serve as an effective therapeutic strategy to cure cancer.

Keywords: tumor microenvironment, TGF-β, EndoMT, extracellular vesicles

S34-2

p21 activated kinase 4 inhibition protects against liver ischemia/reperfusion injury: Role of Nrf2 phosphorylation

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p21 activated kinase 4 (PAK4), an oncogenic protein, has emerged as a promising target for the development of anti-cancer drugs. However, its role in conditions of oxidative stress is not well understood. We investigated the effects of PAK4 signaling on hepatic ischemia/reperfusion (I/R) injury. We first observed a significant increase in PAK4 expression in the livers of mice and humans during I/R injury. Deletion of PAK4 in hepatocytes, but not in myeloid cells, improved liver damage, as evidenced by a reduction in hepatocellular necrosis and inflammatory responses. Conversely, forced expression of wild-type PAK4 exacerbated the pathological changes. We discovered that PAK4 directly phosphorylates Nrf2 at T369, leading to its export from the nucleus, proteasomal degradation, and impairment of antioxidant responses in hepatocytes. The protective effects of PAK4 deficiency were abolished when Nrf2 was silenced in the liver. Additionally, a newly developed PAK4 inhibitor demonstrated protection against hepatic I/R injury in mice. These findings suggest that PAK4 inhibition holds promise as a potential intervention for liver injury induced by I/R.

Keywords: PAK4, Nrf2, ischemia/reperfusion, liver, oxidative stress

S34-3

Vascularized tumor organoid platform for implementing precision oncology for gastric cancer

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Gastric cancer (GC) is the third most common cancer related lethality worldwide; however, unlike other common cancer types, only Her2 status is used clinically to make decisions on sequential lines of treatment. Since Her2 (+) GC is only 15~20% of advanced cancer, VEGFR2 inhibition is clinically accepted as an important therapeutic strategy for Her2 (-) GC. Effective and predictive treatment is pivotal in implementing precision cancer medicine. Given that there is no available predictive biomarker for VEGFR2 inhibitor, it is necessary to develop an alternative method to assess and predict the treatment efficacy of VEGFR2 inhibition *a priori*. Furthermore, accurate prediction of treatment response is essential for overcoming intrinsic therapy resistance that results from genetic heterogeneity, varying tumor growth kinetics, and the complex tumor microenvironment. To achieve this goal, there is an urgent need for effective preclinical *in vitro* models that recapitulate the molecular–pathologic features and intricate ecology of native tumors for precision medicine. In this study, we present a vascularized organoid model (VOM) composed of patient-derived gastric cancer organoids (PDOs), perfusable endothelium, and stomach decellularized extracellular matrix that enables the prediction of clinical response to VEGFR2-targeted therapy in gastric cancer patients. The results indicate that VOMs are dependent on the PDO molecular subtype. Moreover, VOMs accurately reproduce the clinically observed responses of patients treated with VEGFR2 inhibitor. Therefore, VOMs represent a valuable platform for providing clinical predictions for personalized testing and potential discovery of therapeutic drugs in various cancers that lack standardized regimens.

Keywords: vascularized tumor organoid, gastric cancer, 3D bioprinting, precision medicine, VEGFR2

S34-4

Reprogramming of T cell exosmes using surface engineering of IL2 induces potent anti-cancer effects

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T cell-derived small extracellular vesicles (sEVs) exhibit anti-cancer effects. However, their anti-cancer potential should be reinforced to enhance clinical applicability. Herein, we generated interleukin-2-tethered sEVs (IL2-sEVs) from engineered Jurkat T cells expressing IL2 at the plasma membrane via a flexible linker to induce an autocrine effect. IL2-sEVs increased the anti-cancer ability of CD8+ T cells without affecting regulatory T (Treg) cells and down-regulated cellular and exosomal PD-L1 expression in melanoma cells, causing their increased sensitivity to CD8+ T cell-mediated cytotoxicity. Its effect on CD8+ T and melanoma cells was mediated by several IL2-sEV-resident microRNAs (miRNAs), whose expressions were upregulated by the autocrine effects of IL2. Among the miRNAs, miR-181a-3p and miR-223-3p notably reduced the PD-L1 protein levels in melanoma cells. Interestingly, miR-181a-3p increased the activity of CD8+ T cells while suppressing Treg cell activity. IL2-sEVs inhibited tumour progression in melanoma-bearing immunocompetent mice, but not in immunodeficient mice. The combination of IL2-sEVs and existing anti-cancer drugs significantly improved anti-cancer efficacy by decreasing PD-L1 expression in vivo. Thus, IL2-sEVs are potential cancer immunotherapeutic agents that regulate both immune and cancer cells by reprogramming miRNA levels.

Keywords: extracellular vesicles, T cells, interleukin 2

S35-1

Voltage-sensing phosphoinositide phosphatase; mechanisms and functions

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VSP is a voltage-sensitive phosphoinositide phosphatase which consists of the two major regions; a voltage sensor domain similar to that of voltage-gated ion channels and a phosphoinositide phosphatase region with similarity to the phosphatase and tensin homolog, PTEN. Membrane depolarization activates enzyme activity against three species of phosphoinositides, PIP3, PI(4,5)P2 and PI(3,4)P2. VSP has also been utilized for making gene-encoded voltage indicator as well as for altering concentration of phosphoinositides reversibly and acutely.
We have recently addressed both biophysical mechanisms and physiological functions of VSPs. We found that mouse VSP regulates PI(4,5)P2 level along sperm flagella to tune its motility. Knockout mice showed remarkably reduced rate of fertilization and abnormal motility

after capacitation. We also found that zebrafish VSP is localized in endomembranes of one class of enterocytes called lysosome-rich enterocytes and VSP-gene knockout fish exhibits lower efficiency of endocytosis in those cells with higher lethality during juvenile maturation.
In this talk, recent studies on biological functions and some biophysical studies on VSP will be presented with some future perspective.

Keywords: ion channel, phosphoinositide, membrane depolarization, endosome, capacitation

S35-2

$PI(4,5)P_2$ activation of proton-activated chloride (PAC) channels

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Extracellular acidification causes the activation of proton-activated chloride (PAC) channel, thereby playing important role in involving in acid-induced cell death and regulating endocytic pathway. Excepting proton, other factors that regulate the opening of PAC channel are largely unknown. Here, we identified that phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) in the plasma membrane plays an important role in regulating the PAC channel activity. The depletion of $PI(4,5)P_2$ levels through Danio rerio voltage-sensitive phosphatase (Dr-VSP) inhibits hPAC1 and hPAC2 channels. This inhibition also appears after the activation of muscarinic receptor, a $G\alpha_q$ protein-coupled receptor, with signaling pathway that hydrolyze $PI(4,5)P_2$ in both transiently and endogenously expression systems. We further confirmed that PAC channels are regulated by $PI(4,5)P_2$, but not PI(4)P and $PI(3,4,5)P_3$. The single point mutation of basic amino acids located at the interface between transmembrane domain (TMD) 2 and C-terminus adjacent to inner plasma membrane significantly attenuates PAC channel activity. Docking simulation based on Cryo-EM structure reveals that the putative $PI(4,5)P_2$-interacting sites of PAC channel become close to the cell membrane owing to conformational change in activated state, increasing the possibility to bind to $PI(4,5)P_2$. Mutants on $PI(4,5)P_2$-binding sites reduced acid-induced cell death due to suppressed PAC channel activity. Our study proposes that membrane $PI(4,5)P_2$ is important key factor understanding PAC channel gating.

Keywords: PIP2, proton-activated chloride channel, muscarinic receptor, PAC channel, Dr-VSP

S35-3

Lipid scrambling by TMEM16 scramblases

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Lipid scrambling is a rapid process that dissipates the asymmetrical distribution of phospholipids in the plasma membrane. It is involved in various physiological functions such as blood coagulation, apoptosis and viral infection. Many TMEM16 members are recognized as Ca^{2+}-activated phospholipid scramblases, which transport phospholipids between the two leaflets of the plasma membrane nonspecifically and bidirectionally. A membrane-exposed hydrophilic groove in these proteins serves as a shared translocation pathway for ions and lipids. However, the mechanism by which lipids gain access to and permeate through the groove remains poorly understood. Here, we combined quantitative scrambling assays and molecular dynamic (MD) simulations to identify the key steps regulating lipid movement through the groove. We also found that the mechanism of a conformational transition of the groove from membrane-exposed to occluded structure involves the repositioning of transmembrane helix 4 (TM4) following its disengagement from a TM3/TM4 interaction interface. Additionally, we investigated the effects of membrane thickness, lipid composition and the cholesterol on the scrambling activity of TMEM16 proteins. In thicker membrane, TMEM16 scramblase could not transport lipid even in the presence high Ca^{2+} concentration. In the presence of high concentration of cholesterol, TMEM16 protein could not transport lipid. Finally, we investigated the scrambling activity of human TMEM16C protein which is known to be involved in neurological disorder and pain processing. We conclude that the N-terminus of TMEM16C determines whether TMEM16C can translocate to the plasma membrane and facilitate scrambling activity; membrane-localized TMEM16C could transport phosphatidylserine to the outer leaflet.

Keywords: plasma membrane, phospholipids, lipid scramblase, TMEM16, lipid asymmetry

S35-4

Co-imaging lipids and proteins on the plasma membrane by TOF-SIMS reveals increased localization of amyloid precursor protein on lipid rafts in AD neurons

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Lipid rafts on the plasma membrane are thought to provide a platform regulating the activity of signaling pathways by increasing the expression or proximity of proteins in the same pathway. However, lack of direct and simultaneous observation of lipid rafts and associated proteins hamper the elucidation of their roles in various contexts. Amyloid beta (Ab), a hallmark of Alzheimer's disease (AD), is generated upon the sequential cleavage of amyloid precursor protein (APP) by beta- and gamma-secretases. Although this process was known to mostly occur in endosomes in which pH is low enough to activate beta-secretase, recent studies reported increased localization of APP on lipid rafts where beta- and gamma-secretases are enriched in neurons from mouse or human models of AD. In this study, we developed the protocol for co-imaging cholesterol, enriched on lipid rafts, and APP in human induced pluripotent stem cells (hiPSCs) carrying AD-causing variants with the time-of-flight secondary ion mass spectrometry (ToF-SIMS). We first confirmed that lipid rafts could be visualized using quantum-dot-labeled flotillin-1, a major integral protein of lipid rafts in hiPSC-derived neurons. We then found that the levels of extracellular cholesterol could affect the area of lipid rafts and APP localization to lipid rafts. We further elucidated that ApoE4, the strongest risk factor for late-onset AD, induces lipid rafts expansion potentially facilitating beta- and gamma-secretases-dependent APP processing and increasing Ab_{42} synthesis in neurons. This study provides a novel strategy to target ApoE4-dependent amyloidosis by regulating lipid rafts in neurons.

Keywords: lipid rafts, ToF-SIMS, cholesterol, alzheimer's disease, APOE4

S42-1

Phase II study of safety and efficacy of selumetinib in Korean patients with NF1

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Neurofibromatosis type 1 (NF1; OMIM#16220) is a rare genetic disorder affecting 1 out of 3,000 people. NF1 is caused by a heterozygous loss-of-function mutation in the NF1 gene that regulates the RAS-MAPK pathway, which is essential for cellular growth, proliferation, and differentiation. Up to 50% of patients with NF1 develop plexiform neurofibroma (PN), which has the potential for malignant transformation and causes significant morbidity. Oral MEK inhibitor selumetinib decreases the volume of plexiform neurofibroma (PN) in patients with NF1. We evaluated the safety and pharmacokinetic properties of selumetinib along with its efficacy in 89 Korean patients. A total of 89 patients (59 children, 30 adults) received selumetinib for 6-24 months (median, 1.5 yrs). Partial response (≥ 20% volume reduction) was observed in 81 (91%) patients. In addition, improvement was observed in neurocognitive function scores, height score and growth velocity, and Café-au-lait spots intensity as well as in quality-of-life and pain scores. The mean C_{max} and $AUC_{0\text{-}12hr}$ of Korea patients who received a 25 mg/m^2/dose were higher than those in Caucasian children in prior trials. In conclusion, selumetinib may have a broad therapeutic role in NF1, including tumor shrinkage and improvements in neurocognitive function, overall growth, and cutaneous manifestations. This research was supported in part by an externally sponsored research program (ESR-17-12847) by AstraZeneca (provision of selumetinib and funding for study), in collaboration with Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc. (Rahway, NJ, USA).

Keywords: neurofibromatosis type 1, selumetinib, efficacy, therapeutic role, Phase II study

S42-2

Development of a precisional and stratified therapeutic strategy for ALS

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The recent identification of novel mutant genes in ALS and the emerging concept of multisystem proteinopathies have changed the previous concept that ALS affects only the motor neuron system. ALS's clinical and genetic/biological complexities have hindered the development of effective therapeutic strategies for several decades. Current therapeutic strategies for intractable ALS have recently moved to as follows: (1) precision medicine based on genetic characteristics. Precision medicine is an innovative approach that uses emerging biomedical technologies to deliver optimally targeted and timed interventions customized to the molecular drivers of an individual's disease. (2) stratified therapeutic strategy based on clinical characteristics and biomarkers. Even in sporadic patients, the clinical characteristics are not similar and very heterogeneous. Therefore, patients should be subclassified by clinical manifestations and surrogate biomarkers. In the first part, the genetic characteristics of Korean ALS patients will be presented by comparing those of Europeans. And ongoing international clinical trials using ASO precision treatment targeting *SOD1*, *FUS*, *and ataxin-2* mutations will be introduced. The second part will present the necessity of stratified medicine based on biomarkers focused on microglial function. After identifying the different patterns of transcriptomics of induced microglia between rapidly progressing ALS and slowly progressing ALS patients, a recently identified new biomarker, miRNA-214, related to phagocytic dysfunction in ALS will be presented. Identification of novel genes, gene modifiers and biomarkers has expanded our understanding of ALS. With international networks of genetic-clinical dB and Biobank, personalized/stratified prospective therapeutic strategies based on new ALS genes and biomarkers can help us conquer the intractable disease ALS.

Keywords: amyotrophic lateral sclerosis (ALS), gene, precision medicine, biomarker

S42-3

Therapeutic effect of novel chemical inhibitor for RKIP and TbetaR1 binding on NF2 syndrome

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Rational: Neurofibromatosis type 2 (NF2) is autosomal recessive rare genetic disorder, characterized by multiple benign tumors such as schwannomas, meningiomas, and ependymomas. The estimated incidence of NF2 is 1 in 33,000 people worldwide with a penetrance of 95%; however, surgical intervention is the only available treatment.
Genetic cause of NF2 is deletion of NF2 gene, which encoding the merlin. Previously, we revealed that NF2 is required for TβR2 stability. Thus, under the NF2 deficient condition, TβR1 shows relatively higher expression than TβR2 and TβR1 alone can phosphorylate RKIP, resulted in degradation. Since RKIP is important tumor suppressor and inhibitor of Raf/MAPK signaling, NF2 deletion lead to activation of oncogenic pathway. Actually, TEW7197, chemical inhibitor of TβR1 kinase, could induce RKIP expression in NF2 deficient Hei-193 cell and showed the anti-tumor effect on NF2 model mouse. However, TEW7197 also block the canonical TGF-β pathway. Thus, to handling of NF2, selective inhibitor on TβR1-RKIP pathway should be developed.
Hypothesis: Selective RKIP-TβRI binding inhibitor reduces NF2-deficient schwannoma growth without side effects.
Aim: This research was conducted to develop a novel treatment for neurofibromatosis type 2 as well as NF2-deficient MPNST.
Results and Discussion: To obtain the selective inhibitor of TβR1-RKIP, we generated new chemical library and performed the screening based on RKIP expression monitoring system. From the chemical screening and optimization, we finally selected PRG-N-01 as drug candidate. This chemical could induce RKIP expression in NF2 deficient cells without cytotoxicity in normal cells. Moreover, PRG-N-01 did not disrupt the canonical TGF-β pathway. We could also observe the tumor suppressive effect on NF2 model mouse (*Postn*-Cre^{+};NF2$^{fl/fl}$ mouse). Notably, RKIP, induced by PRG-N-01, obviously suppressed the SOX2 expression through nuclear exportation and degradation. In addition, PRG-N-01-induced RKIP expression also regulates expression of SOX10, a crucial transcription factor in Schwann cell differentiation. Indeed, our chemical could suppress the cell cycle related gene set and induced schwann cell differentiation related gene set. Our pre-clinical study showed that PRG-N-01 did not show severe toxicity and very high bio-availability (over than 70%). Considering our investigation, PRG-N-01 would be very reasonable and putative drug for NF2 syndrome. We are now submitting IND to KFDA and planning to clinical trial with NF2 patients in Asan Medical Center.

Keywords: NF2 syndrome, TbR1, RKIP, PRG-N-01, clinical trial

S41-1

Reconstitution of human axial development in vitro

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Various aspects of early embryonic development have been extensively studied using model organisms such as mouse, chick or zebrafish, but remain largely elusive and poorly understood when it comes to human and other primates. Using induced pluripotent stem cell (iPSC)-derived presomitic mesoderm (PSM), we and others have previously succeeded to quantify oscillatory activity of the segmentation clock, a molecular oscillator believed to control somitogenesis, a core developmental event during which the segmented body plan is laid out in all vertebrates. Interestingly, this *in vitro* model of the segmentation clock did not show any sign of segmentation despite the presence of oscillatory activity of clock genes such as *HES7*. Extending on these earlier findings we then asked whether we could recapitulate not only the clock but also the actual process of segmentation and epithelial somite formation *in vitro*. Utilizing again pluripotent stem cells as starting material we established an *in vitro* 3D model of human somitogenesis, which exhibited periodic formation of properly patterned epithelial somites in synchrony with the segmentation clock. Our selforganizing 'axioloids' shared morphological and molecular features of the human embryo and the emerging vertebrate axis including presence of opposing morphogen gradients. We further demonstrated a critical role of retinoic acid (RA) signalling in the stabilisation of segments, suggesting an unexpected synergistic role for RA and ECM in the formation and epithelialisation of somites. Lastly, we applied our bottom-up model system to study the pathogenesis of human congenital diseases of the spine, using patient-like iPSC cells with mutations in *HES7* and *MESP2*, which revealed disease-associated phenotypes including loss of epithelial somite formation and abnormal rostrocaudal patterning. These results suggest that axioloids represent a promising novel platform to study early embryonic development and disease in humans.

Keywords: axioloids, in vitro embryo model, somitogenesis, axial development, disease modeling

S41-2

Human assembloids to study the basic principle of human diseases

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Current technology for human organoids is limited in that it fails to provide essential patterning cues and adequate signals to induce the complexity of the mature human tissues. Recently, we created "human assembloids" derived from two vital tissues in our body, brains and epithelial tissues, and established novel conceptual framework to overcome the major limitations of current organoid technology. These assembloids exhibit the characteristics of mature tissues in the context of cell compositions at the single-cell transcriptome level, and recapitulate the in vivo tissue dynamics and complex cellular interplays during the various physiological and pathological conditions. By developing patient specific mix-and-match human assembloids combined with integrated genetic and epigenetic analysis, our recent works further provided new mechanical insights into the development of various human diseases from cancers to neurological diseases, which is controlled by complex interaction between multiple cell types at distinct developmental and pathological stages in human tissues. Our study also proposed an innovative preclinical model system to study a range of human diseases, whose understanding of pathogenesis requires an organoid system that is capable of representing mature characteristics of functional human tissues with multiple cell types.

Keywords: human assembloid, human organoid, cancer, neurological disease

S41-3

The role of mTOR-S6K1 signal pathway in human brain development and disease

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The physiological functions of S6K1 have been extensively studied in S6K1-deficient mice. However, there is limited evidence demonstrating the influence of S6K1 depletion on human brain development. In this study, we employ genetically engineered human embryonic stem cell-derived brain organoids to identify the role of S6K1 in human brain development. CRISPR-based depletion of S6K1 significantly decreases the size of the dorsal forebrain organoids in the early stages. Morphological changes, including the emergence of neuroectodermal buds, layer separation of progenitors, and maturation of neurons, are also impaired in S6K1 knockout organoids. Moreover, single-cell RNA sequencing analysis shows an abnormal emergence of retinal cell lineages in S6K1-depleted brain organoids, which leads to a decrease in the number of cortical neurons. The brain organoids derived from co-cultured S6K1-depleted and wild-type hESCs show that the roles of S6K1 during brain development are divided into cell-autonomous and non-cell-autonomous functions. Furthermore, we have established a disease model for hyperactivation of mTOR-S6K1 signaling. In this model, an increase in the size of brain organoids was observed in contrast to S6K1 depletion, along with the alteration in differentiation processes. Our research findings provide evidence that mTOR-S6K1 signaling finely tunes the neuronal lineage specification during brain development.

Keywords: brain organoid, mTOR-S6K1, Disease Model

S41-4

Translational application of salivary gland stem cell-derived organoids

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The advent of human 3D organoid systems allows remarkably detailed observation of stem cell morphogens, maintenance, and differentiation that resemble primary tissues, enhancing the potential to study both human physiology and developmental stage. As they are similar to their original organs and carry human genetic information, organoids derived from patients hold great promise for biomedical research and preclinical drug testing. They are currently used for precision regenerative medicine, gene repair, and transplantation therapy. Adult stem cells from salivary glands can be expanded long-term in vitro as three-dimensional organotypic structures termed organoids. These adult salivary glands' stem cell-derived organoids retain their organ

identity and remain genetically stable over long periods of time. The ability to grow organoids from patient-derived healthy and diseased tissue allows for studying organ development, tissue homeostasis, and disease in the era of precision medicine. This lecture shows the generation of adult stem cell-derived organoid cultures and their applications in precision medicine, in vitro disease modeling, drug screening, and regenerative medicine.

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Keywords: Salivary gland, Stem cells, Organoids, Translation, Modeling

S43-1

Cryo-EM structure of amino acid transporter LAT1 and related amino acid signaling downstream of LAT1

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Amino acid transporters are membrane proteins essential for supplying amino acids to cells. They are involved in various pathologies, depending on the amino acids they transport and the cells in which they are expressed. LAT1 (L-type amino acid transporter 1; SLC7A5), specifically expressed in cancer cells, plays a crucial role in cancer cell metabolism. Its expression is upregulated due to metabolic reprogramming in cancer cells, making it an attractive molecular target for cancer diagnosis and therapy. Crystal structure analysis of mammalian transporters has been challenging, with only a few successful cases. However, recent advances in cryo-electron microscopy (cryo-EM) analysis technology have revolutionized the structural analysis of these transporters. The three-dimensional structure of LAT1 has been elucidated using cryo-EM, taking advantage of its heterodimeric structure. This analysis revealed amino acid residues crucial for substrate recognition and conformational changes associated with transport. The ligand-binding structure of LAT1 has also been resolved, providing valuable data for understanding its inhibitory mechanism. The mechanism of LAT1 upregulation in cancer cells has been determined, showing that the oncogene Myc induces LAT1 expression, and ATF4 further induce it as amino acid consumption increases. Comprehensive phosphoproteomics studies of LAT1 inhibitors' effects have revealed strong suppression of cell cycle-promoting kinase pathways, such as those typified by CDKs, indicating that LAT1 inhibitors possess potent cell cycle inhibitory effects. Moreover, LAT1 inhibition results in general translational repression through the mTORC1 and GAAC (general amino acid control) pathways, thereby suppressing the production and secretion of proteins such as cytokines. Consequently, LAT1 inhibitors are thought to exert a wide range of effects, suppressing the functions of cells expressing LAT1 and impacting the surrounding cellular environment. The understanding of LAT1 downstream signaling revealed by inhibitors and the structural insights provided by cryo-EM analysis have clarified LAT1's significance in cancer cell metabolic reprogramming. This knowledge paves the way for developing novel therapeutic agents targeting this transporter.

Keywords: transporter, amino acids, signaling, cryo-EM, cancer

S43-2

A plasma membrane PtdSer subdomain specified by E-Syt3 is essential for assembly of signaling complexes at MCS that activate the HCO_3^- transporters CFTR and NBCe1-B

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The E-Syts (Extended Synaptotagmins) are a family of three ER/PM tethers that regulate the ER-PM junctions. The E-Syts mediate lipid exchange between the ER and PM and affect key organellar communication at membrane contact sites (MCS). Their role in targeting transporters to MCS and regulating their function to coordinate epithelial secretion remains elusive. This presentation will be focused on the specific role of E-Syt3 in the function of luminal CFTR and basolateral NBCe1-B HCO_3^- transporters. E-Syt3 inhibits the activity of these transporters that requires the lipid transfer function of Esyt-3 and its localization in a specific ER/PM junctional nanodomain. The regulation by E-Syt3 was traced to change in plasma membrane PtdSer level and PtdSer sensing by the transporters. PtdSer acted by regulating formation of complexes of the transporters with their signaling pathways. Manipulation of E-Syt3 in salivary glands will be used to determine its potential *in vivo* function.

Keywords: plasma, membrane

S43-3

Cargo translocation on the ERGIC in unconventional secretion

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Protein secretion is a fundamental cellular function for controlling intercellular communications. Traditional protein secretion refers to the classical pathway in which a secretory protein with a signal peptide are recognized by the SRP-SEC61 translocation system and secreted through the ER-Golgi trafficking system. Importantly, multiple secretory proteins without a signal peptide are revealed recently. They are released through unconventional secretion and regulates multiple key physiological processes such as inflammation, metabolism and development. Nonetheless, how these unconventional secretory proteins are delivered out of the cell has been a mystery in the field, especially how proteins without a signal peptide can be targeted to the secretory destination. Here we identify the TMED family proteins as key protein channels for regulating multiple unconventional secretory proteins entry into a vesicle carrier, the ER-Golgi intermediate compartment (ERGIC), as an initial step regulating the secretion of unconventional secretory proteins. In addition, we identified specific lipid composition on ERGIC which maintains the activity of TMEDs, and therefore defines the ERGIC as the membrane carrier, instead of the ER(which is for classical secretion), for unconventional cargo entry into the trafficking system. Finally, different members of the TMED control cargo specificity which likely accounts for how cells handle the specific release of a variety of unconventional secretory cargoes.

Keywords: TMED, protein

S43-4

Unconventional proteins secretion of CFTR, Pendrin, and SARS-CoV-2

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Most eukaryotic secretory or membrane proteins reach the plasma membrane through the conventional endoplasmic reticulum (ER)-Golgi pathway. However, evidence suggests that many cytoplasmic, nuclear, and signal-peptide-containing proteins also can reach the cell surface via a Golgi-bypassing route. These pathways are collectively referred to as unconventional protein secretion (UPS). A number of transmembrane proteins, including CFTR, CD45, Kv4, Mpl, pendrin are SARS-CoV-2 Spike, are known to reach the cell surface via UPS under specific conditions. Understanding the UPS pathways is important not

only to elucidate the mechanisms of intracellular trafficking pathways, but also has important ramifications for human health because many proteins undergo UPS are associated with human diseases. For example, our group recently found that the selective activation of the UPS pathway would be a potential therapeutic strategy for the treatment of diseases arising from the transport defects of misfolded proteins, such as cystic fibrosis and Pendred syndrome. In contrast, the inhibition of TMED3 complex-mediated UPS reduces SARS-CoV-2 viral release. In this talk, I will introduce the molecular machineries involved in the UPS of transmembrane proteins, particularly those related to human diseases, and discuss the potential for new therapeutic strategies for the treatment of UPS-associated diseases.

Keywords: unconventional secretion, CFTR, pendrin, SARS-CoV-2, TMEDs

S44-1

Investigation of the enhancive role of NLRP3 inflammasome for neutrophil infiltration to the inflamed mouse brain

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In response to systemic inflammation, neutrophils rapidly exit the vascular compartment across the blood-brain barrier (BBB) and migrate into the inflamed brain parenchyma. Recently, it has been reported that neutrophil infiltration to brain parenchyma tissue exacerbates brain tissue and nerve damage. However, the detailed molecular mechanism of neutrophil infiltration to the brain tissue is still elusive. In this study, we pursue to identify the molecular mechanisms of NLRP3 inflammasome, which plays a pivotal role in neutrophil migration within the inflamed mice brain induced by LPS administration. We identified that NLRP3 inflammasome activation in brain tissue dramatically elevated the number of infiltrated neutrophils to inflamed-brain parenchyma. Using two-photon intravital of mice brain, we also found that NLRP3 inflammasome increased brain vascular permeability. In addition, it is still going on to explore critical molecules in the BBB, which are involved in these phenomena. To further investigate how NLRP3 activation in neutrophil affects infiltration of these cells to inflamed-brain tissue at the molecular level, we generated mutant mice with neutrophil specific expression of constitutively active NLRP3. Interestingly, the NLRP3-active mutant mice have an augmented number of brain-infiltrated neutrophils even in the absence of LPS stimulation. Furthermore, the NRLP3-active mutant mice showed a decreased expression level of specific molecules in the BBB in brain tissue, along with an increased expression level of CXCL1 and CXCL2 in the neutrophils. In summary, our findings reveal that the NLRP3 activation in neutrophil is critical for both neutrophil infiltration to inflamed brain tissue and BBB breakdown.

S44-2

Two-Photon microscopy imaging of synaptic structures, neuronal/glial calcium and cerebrospinal fluid in living mice

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Recent advances in two-photon microscopy, fluorescence labeling techniques and genetically encoded calcium indicators have enabled us to directly record the structural and functional changes in neurons and glia, and even at synapses, in the brain of living animals. Long-term *in vivo* two-photon imaging studies have shown that some postsynaptic dendritic spines in the adult cortex are rapidly eliminated or newly generated, in response to altered sensory input or synaptic activity, resulting in experience/activity-dependent rewiring of neuronal circuits. In vivo two-photon Ca^{2+} imaging studies have revealed the distinct, input-specific response patterns of neurons and astrocytes in the brain. Furthermore, intracisternal injection of fluorescent dyes or microspheres with two-photon microscopy could visualize and quantify the flow of cerebrospinal fluid in real time, which reflects a function of the glymphatic system. These updated *in vivo* approaches are now being widely used for the study of pathophysiological mechanisms of neurological diseases. In this talk, I will introduce my previous and ongoing works in the last decade, focusing on *in vivo* two-photon microscopy imaging of synaptic structures, glia/neuronal calcium and cerebrospinal fluid in living mouse brain for research in various fields of neuroscience.

Keywords: two-photon microscopy, synapse, glia, neuron, cerebrospinal fluid

S44-3

Holographic microscope for multi-cellular measurement and manipulation

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The interaction of neuron and glia is essential for functional neuronal circuits. We have been especially focused on microglia, a sole immune cell in CNS. In addition to the pathological function of microglia, recent developments in molecular probes and optical imaging *in vivo* have revealed that microglia are highly motile cell in the healthy brain, extending and retracting their process that extend from a largely stationary cell soma. We reveal their physiological and pathological function on synapse and vessels. In this session, we will show microglial role for cross modal plasticity and show how they can contribute on sensory discrimination by their effect on neural circuit function. In addition, we will introduce our recent developed holographic microscope that can precisely measure and manipulate neuron and glial cell activities in a spatiotemporal manner in living mice to ultimately affect on behavior output. Furthermore, we will show our successful evaluation method to analyze the functional neuronal connectivity to integrate the transcriptome information.

Keywords: holographic microscope, microglia, in vivo two photon microscope

S44-4

Getting sharper: super-resolution imaging of dynamic brain microstructures

Valentin Nägerl

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Brain cells such as neurons and astrocytes exhibit an extremely elaborate morphology, and their functional specializations like synapses and glial processes often fall below the resolution limit of conventional light microscopy. This is a huge obstacle for neurobiologists because the nanoarchitecture critically shapes fundamental functions like synaptic transmission and Ca^{2+} signaling. Super-resolution microscopy can overcome this problem, offering the chance to visualize the structural and molecular organization of brain cells in a living and dynamic tissue context, unlike traditional methods like electron microscopy or atomic force microscopy. In my talk I will review our contributions to developing live-cell super-resolution (STED) microscopy approaches and their application to key problems in cellular neurobiology concerning the structure, function and plasticity of hippocampal synapses and the surrounding extracellular space.

Keywords: two-photon STED microscopy, super-resolution shadow imaging, extracellular calcium imaging, dendritic spines, tripartite synapses

S45-1

Functional role of detrusor interstitial cells during bladder filling

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As the bladder fills with urine the volume increases and the walls are stretched, but during much of the filling period, intravesical pressure remains low. This accommodation occurs even though there is a natural tendency for the detrusor smooth muscle cells to contract in response to stretch. In fact, during bladder filling non-voiding contractions (NVCs), detected as transient increases in intraluminal pressure, occur in cystometric records from all species including human. NVCs appear to correspond to localized contractions that are also observed in ex vivo bladder preparations and have been termed 'spontaneous phasic contractions', 'micromotions' or 'transient contractions'. Transient contractions (TCs) increase as bladder filling proceeds. Normal bladders have the means to restrain development of TCs, but previous experiments have not been sufficiently rigorous to reveal the mechanisms responsible for restraining bladder excitability and the development of TCs during filling. Evidence suggests that development of TCs during filling is independent of neural input because these contractions: i) persist in ex vivo bladders disconnected from central reflexes, and ii) they are not affected by neurotoxins that block nerve action potentials. We discovered a novel mechanism of bladder filling involving interstitial cells in detrusor muscles. The interstitial cells in the detrusor layer are platelet-derived growth factor receptor alpha positive (PDGFRα^+), and they provide inhibitory regulation of detrusor muscle excitability. Inhibitory regulation is enhanced by purines and by stretch, making it an ideal mechanism for control detrusor excitability and restraining TCs during bladder filling. An important next step in understanding the significance of PDGFRα^+ cells in regulation of bladder excitability would be to discover pathological conditions in which development of DO occurs with loss or remodeling of PDGFRα^+ cells. Loss of mechanisms that control detrusor excitability, as proposed in the myogenic hypothesis, might lead to NVCs. Spinal cord injury (SCI) induced DO, but a mechanism for DO after SCI has not been determined. We have discovered significant damage to PDGFRα^+ cells following SCI, an animal model that is known also to develop DO. This exciting observation might provide a novel explanation for SCI-induced DO.

Keywords: bladder smooth muscle, interstitial cell, platelet-derived growth factor receptor alpha positive, PDGFRα^+, voiding disorder

S45-2

Functional and morphological abnormalities of Interstitial Cells of Cajal (ICC) in gastrointestinal disorders

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The functional and morphological abnormalities of ICCs and their molecular mechanisms in gastrointestinal disorders will be presented. We established an *in vitro* functional assay for ICCs by generating small intestinal organoid clusters containing almost component of the muscle layer, such as smooth muscle cells, intramural nerves, resident macrophages, ICCs, and PDGFRα positive mesenchymal cells (Pα cells). The pacemaker function of ICCs was unaffected by IL-1β, IL-6, TNF-α, INF-γ, and LPS, but was markedly suppressed by simultaneous INF-γ and LPS exposure. The inhibition was abolished by L-NAME and Apocynin treatment, indicating that the function is impaired by reactive oxygen species (ROS; ONOO-) released from NO. The results obtained with the organoid were also validated in a postoperative ileus (POI) model of mice. There was the dysfunction of ICC pacemaker 24 hours after mechanical manipulation of the small intestine in POI, and the dysfunction was abolished by aminoguanidine treatment. C-kit-positive ICC cells disappeared 24 hours after the mechanical treatment, but c-kit-positive ICCs with pacemaker ability reappeared 48 hours after manipulation, indicating that ICCs are highly plastic cells, which can easily change into c-kit negative ICCs without pacemaker ability by active products. Next, we examined gastrointestinal motility in diabetic gastroenteropathy, using streptozotocin-induced type 1 diabetes model (DM). The upper and lower gastrointestinal motility is hyperreactive or hyporeactive in DM, with increment or decrement in ICC network, respectively. The ICC pacemaker function monitored by microelectrode was also enhanced in the upper gastrointestine. This opposite phenomenon in response to oxidative stress via high glucose in DM was attributed to the increased expression of the antioxidant enzyme heme oxygenase-1 (HO-1) in the upper gastrointestinal tract, which contributes to the protection and proliferation of ICCs. HO-1 induce transformation of M1 to M2 macrophages, resulting in releasing PDGF and possibly activating Pα cells. The activated Pα cells produce SCF to activate the c-kit receptor, which induces ICC proliferation. In conclusion, in gastrointestinal diseases, the generation of oxidative stress/ ROS in the gastrointestinal tract and its protective state cause the decrease or increase of ICC network, which is involved in the dysmotility of the gastrointestinal tract, such as the suppression or enhancement of gastrointestinal motility. These findings will be helpful in the development of new gastroprokinetic agents targeting ICC.

Keywords: gastointestine, interstitial cells of Cajal (ICC), c-kit, reactive oxygen species (ROS), diabetes

S45-3

Ion channels for the spontaneous rhythm and contractility regulation in colonic interstitial cells of cajal

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Background: Interstitial cells of Cajal (ICC) are pacemaker cells and play an important roles in the regulation of smooth muscle activity by producing and propagating slow waves, transducing neural input to smooth muscles, and acting as stretch receptors in the gastrointestinal (GI) tract. Many neurotransmitters and hormones affect the frequency and configuration of slow waves, thus regulating GI motility. ICC generate spontaneous pacemaker potentials that correlate with peristaltic contractions in the small intestine. The pacemaking mechanism has been well studied in cultured ICC and intact mouse small intestine. The coupling between IP_3-gated Ca^{2+} release from endoplasmic reticulum and reuptake of Ca^{2+} into mitochondria is linked to generation of pacemaker activity by periodically activating Ca^{2+}-dependent membrane pacemaker ion channels. Transient receptor potential channels or Ca^{2+}-activated Cl^- channels (ANO-1) were candidates as pacemaker channels in ICC. The pacemaker mechanisms within GI tracts are believed to be similar, but key differences do exist. The frequency of slow waves varies markedly in the different GI organs, and also differs between species. There are also differences in ion channel function between organs as L-type Ca^{2+} channels appear to play an important role in slow wave generation in the colon. Its difference of slow waves between small intestine and colon provides the possibility that the different ion channel operation in pacemaker activity can be exist in colonic ICC. Therefore, we studied pacemaker activity between small intestinal and colonic ICC and compared it.
Methods: Electrophysiological, immunohistochemical, and molecular techniques were used in mouse colon.
Results: We found that periodically activated hyperpolarization-activated cyclic nucleotide (HCN) channels by basal intracellular cAMP production are present in colonic ICC and they serve as a pacemaker channel. Together with, ANO-1, voltage-dependent Ca^{2+}, and ATP-sensitive K^+ channels were participated in generating pacemaker potentials. The different regional distribution in HCN channels between small intestine and colon suggest a cause for differential regulation of GI motility.
Conclusion: Therefore, HCN channel may participate in regulation of pacemaking activity and may be an effective therapeutic target for abnormal colonic motility disorders.

Keywords: interstitial cells of Cajal, colon, pacemaker potential, HCN channel, GI motility

S45-4

Contribution of the Na^+-leak channel NALCN to arterial contractility in health and disease

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Na^+-leak channel NALCN reportedly contribute to the neuronal excitability in health and disease. However, roles of NALCN in the arterial contractility is not known well. In this study, we examined the contribution of NALCN to the increased vasoconstrictions observed in the hypertensive animals. Altered salt homeostasis is one of major risk factors of hypertension. Although the increased renal Na^+ retention and intravascular volume overload by altered activity of mineralocorticoid (MC) hormone in kidneys contribute to hypertension, a non-renal mechanism, likely related to the vasculature, is also involved. In this study, we examined the non-renal hypothesis that increased functions of NALCN in arterial smooth muscle contribute to the increased vasoconstriction of MC (deoxycorticosterone acetate, DOCA)-salt hypertensive rat. Membrane currents and membrane potential (*Em*) were measured by nystatin-perforated patch-clamp technique. Isometric vasoconstriction was measured from mesenteric arterial rings. Effects of extracellular Na^+ reduction and pharmacological blockers of Na^+-permeable channels such as flufenamic acid (FFA) and amiloride/benzamil were examined. Protein expression of NALCN in the arteries were examined with western blotting. The basal tone and serotonin (5-HT)-induced vasoconstrictions were substantially increased in DOCA-salt hypertensive mesenteric arteries. Comparison of membrane conductance (*Gm*) of sham and DOCA-salt hypertensive arteries indicated that resting *Gm* was lager and that *Gm* increased even more in response to 5-HT in DOCA-salt hypertensive arterial smooth muscle than in sham arterial smooth muscle. Reduction in $[Na^+]_{out}$ normalized the increased vasoconstriction of the DOCA-salt hypertensive arteries as well as normalizing both the depolarized resting *Em* and the exaggerated 5-HT-induced *Em* depolarization. Effects of FFA were similar to those of low $[Na^+]_{out}$. However, ENaC blockers amiloride and benzamile were without effect. Protein expression of NALCN was significantly enhanced in the DOCA-salt hypertensive arteries. Furthermore, knock-down of NALCN normalized the increased arterial constrictions observed in the hypertensive subjects. These results indicate that the enhanced activity of the NALCN in DOCA-salt hypertensive rat arterial smooth muscle critically contribute to the hyper-reactivity of DOCA-salt hypertensive rat.

Keywords: hypertension, mineralocorticoid, ENaC, NALCN, vascular smooth muscle

S46-1

Impairment of autoinhibition in locus coeruleus neurons is involved in the pathogenesis of Alzheimer's disease

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The earliest pathogenesis of Alzheimer's disease (AD) occurs in the locus coeruleus (LC). Cytosolic free noradrenaline (NA) is metabolized by MAO-A to DOPEGAL, which activates asparagine endopeptidase (AEP) and consequently induces LC degeneration. However, it is unknown how cytosolic free NA is accumulated in the cell body of LC neurons to eventually cause cellular degeneration. Because stress increases spike activities in LC neurons and chronic stress causes plastic changes in LC neurons, we examined whether the autoinhibition mediated by α2A adrenergic receptor (AR)-coupled GIRK currents is impaired by stress or excitability increases, using patch-clamp and immunohitochemical methods. The expression levels of α2A-ARs and GIRK-channels were also examined by western blot/qPCR analyses. Restraint stress (RS) decreased NA-induced GIRK currents while a similar decrease in NA-induced GIRK currents was caused in non-stress mice in a Ca^{2+}- and β-arrestin-dependent manner. In RS mice, the internalization of α2A-ARs and decreases in α2A-ARs/GIRKs in protein/mRNA levels were detected in membrane fraction. Impairment of autoinhibition in RS mice or by $[Ca^{2+}]_i$ increases increased the activities of TH, MAO-A and AEP. Thus, impaired autoinhibition would cause free NA accumulation in the cell body, subsequently increasing AEP activity through MAO-A metabolite to promote LC degeneration.

Keywords: locus coeruleus, α2A adrenergic receptor, GIRK, restraint stress, Alzheimer's disease

S46-2

Neurobiology of capsaicin-induced analgesia for trigeminal neuropathic pain

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Capsaicin, the pungent ingredient in chili peppers, produces intense burning pain in humans. Capsaicin selectively activates the transient receptor potential vanilloid 1 (TRPV1), which is enriched in nociceptive primary afferents, and underpins the mechanism for capsaicin-induced burning pain. Paradoxically, capsaicin has long been used as an analgesic. However, neurobiological mechanisms of capsaicin-induced analgesia is not well understood. High concentrations of capsaicin lead to long-term defunctionalization mediated by the ablation of TRPV1-expressing afferent terminals, resulting in long-lasting analgesia for neuropathic pain in humans and rodents. Recently, we showed that capsaicin-induced ablation of axonal terminals mediated by Ca^{2+}/calpain/microtubule depolymerization is necessary to produce long-lasting analgesia for mechanical hyperalgesia in a mouse model of neuropathic pain. Capsaicin-induced analgesia was also associated with comprehensive changes in brain activities across the regions involved in pain. Longitudinal functional magnetic resonance imaging (fMRI) scanning followed by resting state functional connectivity analysis was conducted in rats to determine the changes in brain activities associated with capsaicin-induced analgesia for neuropathic pain. Trigeminal neuropathy induced functional reorganization of widespread cortical and subcortical regions. Interestingly, the peripheral administration of capsaicin reversed a substantial proportion of such changes, which was correlated with capsaicin-induced analgesia. Furthermore, the fMRI scanning under chemogenetic silencing of the anterior cingulate cortex (ACC), which plays critical roles in sensory and affective neuropathic pain, revealed the specific brain correlates commonly associated with analgesia achieved by both capsaicin and chemogenetic silencing. These studies suggest that peripheral administration of capsaicin can produce long-term analgesia associated with reversal of the pathological brain connectivity. Further determination of the neurobiological mechanisms of peripherally induced analgesia should lead to more efficacious non-opioidergic analgesic options with fewer adverse side effects.

Keywords: capsaicin, neuropathic pain, analgesia, brain imaging

S46-3

A role of central angiotensin II in orofacial pain

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Angiotensin (Ang) II, a peptide hormone, regulates vascular contraction, hormone secretion, and fluid balance. However, peripheral Ang II is not active in the central nervous system, as the blood-brain barrier is impermeable to peripheral Ang-related components. Previous studies have demonstrated that angiotensinogen and angiotensin-converting enzyme (ACE) are found in the cerebrospinal fluid and the brain. These results suggest that Ang II has important functions in the central nervous system in addition to its peripheral effects. Recently, emerging findings indicate that central Ang II contributes to modulation of nociceptive progression in the central nervous system. However, the underlying mechanisms of central Ang II in modulation of nociceptive transmission in the orofacial area remain unclear. The present study showed differential regulation of mechanical allodynia and thermal hyperalgesia in the orofacial area following intracisternal injection of Ang II. In addition, we identified the differential role of ACE 1 and 2 in mechanical allodynia in rats with trigeminal neuropathic pain. Intracisternal administration of Ang II produced both mechanical allodynia and thermal hyperalgesia in naïve rats. Ang II-induced mechanical allodynia was mediated by the angiotensin type 1 (AT1) receptor expressed in astrocytes. The AT2 receptor in the primary afferent terminal regulated Ang II-induced thermal hyperalgesia by modulating release of neuropeptides such as substance P in the terminals of primary afferent fibers. Inferior alveolar nerve injury, which produced significant mechanical allodynia, upregulated the expression of angiotensinogen in the trigeminal subnucleus caudalis. Intracisternal administration of captopril, an ACE 1 inhibitor, and losartan, an AT1 receptor antagonist, attenuated trigeminal neuropathic mechanical allodynia. On the contrary, intracisternal administration of diminazene aceturate, an ACE 2 activator, and angiotensin (1-7) attenuated trigeminal neuropathic mechanical allodynia. We also evaluated changes in ACE 1 and 2 expression in naïve and nerve injured animals. Moreover, intracisternal administration of DX600, an ACE 2 inhibitor, produced nociceptive behavior in naïve rats. These results suggest that central Ang-mediated nociception is differentially regulated by Ang receptors and ACEs. Therefore, specific pathways must be targeted to overcome different types of pain symptoms, as multiple underlying mechanisms contribute to development of chronic pain.

Keywords: angiotensin II, angiotensin II receptor, angiotensin-converting enzyme, neuropathic pain, trigeminal nucleus

S46-4

Role of non-neuronal cells in persistent orofacial pain

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Trigeminal nerve injury frequently occurs following surgical procedures in the orofacial regions, such as tooth extraction or dental implantation, and sometimes causes persistent orofacial pain. However, detailed mechanisms underlying persistent orofacial pain associated with trigeminal nerve injury are not thoroughly elucidated. The neuron-glia interaction within the trigeminal ganglion (TG) and the trigeminal spinal subnucleus caudalis (Vc) is thought to be a possible mechanism for the persistent orofacial pain associated with trigeminal nerve injury. Following trigeminal nerve injury, the injured neurons become hyperactive. The various cytokines, such as IL-1, TNFa, IFNg, neuropeptides, or nitric oxide, are released from neurons and glial cells, and those molecules contribute to neuron-glia communication in TG and the Vc. We recently observed that the IL-33 expression was increased in oligodendrocytes in the Vc following infraorbital nerve injury. Neutralizing the IL-33 receptor in Vc relieved mechanical hypersensitivity associated with infraorbital nerve injury. We also found that the IL-33 expression in the TG was significantly enhanced after infraorbital nerve injury. The immunohistochemical study revealed that IL-33 was expressed in αSMA or CD34 (fibroblast marker) immunoreactive cells, and IL-33 receptor was expressed in TG neurons in trigeminal-nerve injured mice, suggesting that the IL-33 released from fibroblasts after the nerve injury was involved in the hyperactivation of TG neurons. We also observed that intra-TG administration of IL-33 caused mechanical hypersensitivity in the whisker pad skin. These results suggest that IL-33 released from oligodendrocytes causes the enhancement of Vc neuronal activity, and IL-33 released from fibroblasts in TG also causes increased TG-neuronal activity, resulting in persistent orofacial pain following infraorbital nerve injury. In this symposium, we address our current results regarding mechanisms of neuro-glia interaction following trigeminal nerve injury and discuss its contribution to persistent orofacial pain.

Keywords: trigeminal nerve, glia, cytokine, persistent pain, nerve injury

S46-5

Transcriptome profiling of dental sensory system by single-cell RNA sequencing

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Dental primary afferent (DPA) neurons and proprioceptive mesencephalic trigeminal nucleus (MTN) neurons, located in the trigeminal ganglion (TG) and the brainstem respectively, are two main somatosensory neurons which control masticatory functions. Despite transcriptomic studies on various somatosensory neurons, there is still a lack of knowledge about the molecular characteristics of these populations. Here, we address this using high depth single-cell RNA-sequencing (scRNA-seq) combined with retrograde tracing in mice. Our transcriptome analysis revealed five major types of DPA neurons with cell type-specific gene enrichment, some of which exhibit unique mechano-nociceptive properties capable of transmitting nociception in response to innocuous mechanical stimuli in the teeth. In addition, DPA and MTN neurons represented sensory compartments with distinct molecular profiles characterized by various ion channels, receptors, neuropeptides, and mechanoreceptors. Together, our study provides new biological insights with respect to the highly specialized mechanosensory functions of DPA and MTN neurons in pain and proprioception.

Keywords: dental afferents, proprioception, nociception, pain, trigeminal system

S38-1

Autophagy regulation by the intracellular Ca^{2+} channel TRPML3

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Autophagy progresses through Ca^{2+}-dependent multiple fusion events. Recently, we reported that the intracellular Ca^{2+} channel TRPML3 provides Ca^{2+} for autophagosome formation as a downstream effector of PI3P. However, the molecular mechanism of Ca^{2+} signaling in late stage of autophagy remains unknown. Here, we show that TRPML1/3 heteromer is the Ca^{2+} provider for autophagosome-lysosome fusion. TRPML1/3 functions downstream of PI4P to increase autophagosome-lysosome fusion and the Ca^{2+} signal via PI4P-TRPML1/3 is decoded by synaptotagmin 5 (SYT5) Ca^{2+} sensor. Binding of Ca^{2+} and PI4P to SYT5 is critical for autophagosome-lysosome fusion by forming a fusion complex. Collectively, these results reveal that PI4P-TRPML1/3-SYT5 constitute the Ca^{2+} signaling complex in autophagosome-lysosome fusion and that TRPML3 also regulates late stage of autophagy by heteromerization with TRPML1 in a phosphoinositide-dependent manner.

Keywords: autophagy, PI4P, TRPML3, SYT5, Ca^{2+} channel

S38-2

Structural basis of the activation of TRPV5 channels by long-chain acyl-Coenzyme-A

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Long-chain acyl-coenzyme A (LC-CoA) is a crucial metabolic intermediate, which plays important cellular regulatory roles, including activation and inhibition of ion channels. The structural basis of ion channel regulation by LC-CoA is not known. Transient receptor potential vanilloid 5 and 6 (TRPV5 and TRPV6) are calcium-selective ion channels found in epithelial cells. In this study, we demonstrate that LC-CoA activates TRPV5 and TRPV6 in inside-out patches, and it can take

the place of the natural ligand phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) in maintaining channel activity in intact cells. We used cryo-electron microscopy (cryoEM) to determine the structure of TRPV5 bound to LC-CoA. The LC-CoA-bound TRPV5 showed an open configuration, and LC-CoA occupied the same binding site as $PI(4,5)P_2$ in previously determined structures. This finding is consistent with LC-CoA's ability to occlude channel activation by $PI(4,5)P_2$. Our data provide molecular insight into ion channel regulation by a metabolic signaling molecule.

Keywords: Long-chain acyl-coenzyme A, TRPV5, TRPV6

S38-3

Protease-activated receptors regulate excitability of SIP syncytium through different ion channels

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Gastrointestinal (GI) motility occurs through the cooperation of smooth muscle and nerve to mix and migrate contents of digestion. GI smooth muscles are complex tissues composed of various cell types such as interstitial cells, nerve cells, smooth muscle cells (SMC), and immune cells. To date, two types of interstitial cells have been known to be involved in GI motility, interstitial cells of Cajal (ICC) and $PDGFR\alpha^+$ cells. ICC and $PDGFR\alpha^+$ cells are electrically connected to smooth muscle cells to form SIP syncytium. Various types of receptors and ion channels expressed in SIP syncytium regulate GI motility in various ways. Most of the GI disorders have been known to be related to the abnormalities of the cells that make up the SIP syncytium. As a cause of GI dysmotility, not only changes in the number of these cells, but also the alterations in receptors and ion channels distributed in each cell are important. However, the role of these receptors and ion channels for GI motility has not been cleared in the physiological and pathophysiological condition. Protease-activated receptors (PARs) are widely distributed in GI tract and participate in regulation of motility, but little is known about the cells and mechanisms in GI muscles responsible for PAR responses. PAR1, 2, 3, and 4 are expressed in human and murine colonic smooth muscles. PARs induced transient hyperpolarization of colonic muscles, with relaxation responses followed by excitation. The inhibitory phase was mediated by activation of small conductance calcium-activated potassium (SK) channels in $PDGFR\alpha^+$ cells. The excitatory response resulted from activation of a chloride conductance in ICC and activation of small amplitude inward currents and Rho-kinase in SMCs by PAR activation. In inflammatory state of colon, contractile responses by PAR was changed. Downregulation of SK channels and upregulation of Ca^{2+} sensitization pathways mediated the change of contractile responses by PAR. These results will provide the basis for explaining the change in motility in human inflammatory bowel disease (IBD) patients.

Keywords: protease-activated receptors, interstitial cells of Cajal, $PDGFR\alpha^+$cells, smooth muscle cells, GI motility

S38-4

Regulation of CFTR trafficking via cell stress-associated ER structural changes

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Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene resulting in CFTR deficiency can cause cystic fibrosis due to the malfunction of CFTR anion channel activities. The most prevalent disease-causing mutation of CFTR is the deletion of Phe508 (△F508), which disrupts the cell-surface trafficking of CFTR. It has been suggested that the surface expression of △F508-CFTR can be rescued by upregulation of unconventional protein secretion (UPS) pathways. Induction of cellular stresses, such as the blockade of conventional ER-to-Golgi trafficking route or evoking ER stress signals, has been shown to activate UPS. However, the underlying mechanisms of UPS remain largely elusive. In the present study, we found that induction of UPS by ER stress is associated with structural changes in the ER. Induction of CFTR UPS by the blockade of ER-to-Golgi trafficking using the dominant-negative Arf1-Q71L mutant resulted in the formation of ER puncta structures where △F508-CFTR is concentrated. We found out that the ER puncta structures are consist of elongated ER membranes and ER-derived vesicles. Notably, these ER puncta structures are not static and not only dynamically mediate the cellular vesicular trafficking but are closely related with ER-phagy adaptors. Based on our results, we suggest that ER puncta formation is one of the key factors in the cell stress-associated trafficking of CFTR.

Keywords: ER structure, unconventional protein secretion, cell stress-associated trafficking, CFTR, ER-phagy adaptors

S48-1

Development of brain-targeting microRNA-based ASO formulation against neurodegenerative diseases

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Background: The microRNA, miR-485-3p, has been implicated as a major pathway of neuroinflammation leading to neurodegeneration in several diseases. MiR-485-3p is significantly upregulated in animal models and patients with Alzheimer's disease (AD) and further found that suppression of miR-485-3p delayed disease progression and improved cognitive function in the animal models. Further research showed that this neuroinflammatory pathway is also active and pathogenic in amyotrophic lateral sclerosis (ALS) and Parkinson's disease (PD). In parallel, BIORCHESTRA has identified that miR-485-3p can be measured in peripheral blood and saliva, and that miR-485-3p levels correlate with disease activity in the animal models of ALS and PD

and is currently developing an antisense oligonucleotide (ASO)-based therapeutic targeting miR-485-3p and has successfully developed a proprietary drug delivery formulation.
Methods: Expression of miR-485-3p was analyzed by real-time PCR in the human frontal cortex, precentral gyrus, CSF, plasma, and animal tissues. For CNS delivery, we utilized a proprietary non-lipid polyionic nanoparticle, equipped with a surface ligand facilitating blood-brain barrier transport via the LAT1 receptor. Then, having developed nanoparticle containing an ASO targeting miR-485-3p, named BMD-001, we validated brain delivery in sequential animal models up to non-human primates. To investigate the efficacy of BMD-001, various disease model mice including 5XFAD mice for AD, SOD1*G93A mice for ALS, and a-synuclein preformed fibrils (PFF) injected-mice for PD were used. BMD-001 was administered to those mice by intravenous injection, once weekly for four weeks. Behavior and clinical symptoms were evaluated and various endophenotypic pathologies were analyzed.
Results: We have confirmed the efficient ASO delivery into the CNS, especially neuroinflammation-related cells (neurons, microglia, and astrocytes) expressing the LAT1 receptor in both *in vitro* and *in vivo* studies. Levels of miR-485-3p were higher in biofluid or brain tissue samples of AD, ALS, and PD patients compared to healthy controls. Overexpression of miR-485-3p in cultured neurons, glia, or whole animals clearly induced the pathophysiological deterioration known to be either common or specific to those diseases. On the other hand, downregulation of miR-485-3p by the Company's ASO-based therapeutic showed remarkable and beneficial effects on disease progress and activity in specific animal models of AD, ALS, and PD.
Conclusion: Our novel, first-in-class, brain-targeting delivery system demonstrated enhanced CNS delivery of the ASO and significant inhibition of miR-485-3p after IV administration. Our findings suggest that miR-485-3p may be not only a useful biomarker but also a causative factor of neurodegenerative pathophysiology. Furthermore, BMD-001 has promising potential as a candidate for the treatment of patients with neurodegenerative diseases.

Keywords: miR-485-3p, microRNA, ASO, BBB, nanomedicine

S48-2

Dysregulation of RNA binding proteins in ALS

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RNA binding proteins (RBPs) are involved in all stage of the RNA life cycle in both the nucleus and cytoplasm to regulate RNA metabolism including splicing, polyadenylation, editing, transport, translation, and turnover. More than 50% of known RBPs are expressed in the brain, where they are crucial for neuronal ribostasis and play a broad role in normal and pathological brain function. Dysfunctional mutant RBPs found in patients with neurodegenerative diseases results in dysregulation of RNA metabolism in neurons. RBPs are the main components of stress granules (SGs) that interact with each other, and these granules rapidly consolidate under stress conditions. Several disease-causative or -associated RBP mutations involved in SG dynamics, such as TDP-43, FUS, ATXN2, hnRNPA1, and TIA1 have been identified to increase the risk of ALS/FTD. These RBPs can commonly undergo liquid-liquid phase separation (LLPS) to form biological condensates in physiological conditions. During pathogenic processes such as gene mutations, post-translational modifications, stress conditions, and aging, aberrant phase separation behavior (from liquid-liquid to liquid-solid) may trigger to irreversible and toxic aggregation of target disease proteins. In this presentation, I will focus on previously unrecognized functions of *ANXA11* related to intracellular Ca^{2+} homeostasis and stress granule dynamics, two essential cellular mechanisms of emerging interest in ALS/FTD research. We analyzed the exome sequences of 882 Korean patients with sALS and identified 16 variants of *ANXA11* in 26 patients. We show that N-terminus variants within the low complexity domain of ANXA11 enhance aggregation propensity, while C-terminus ANX domain variants alter Ca^{2+} responses. Furthermore, mutations in ANXA11 undergo abnormal phase separation to form droplets with aggregates and lead to the alteration of the biophysical properties of ANXA11. These functional defects caused by ALS-linked variants induce alteration of both intracellular Ca^{2+} homeostasis and stress granule disassembly. Expression of ALS-linked *ANXA11* variants in motor neuronal cells causes cytoplasmic sequestration of endogenous FUS, suggesting toxic gain-of-function. The mechanisms underlying the neurotoxicity induced by aggregated proteins remain controversial. In this presentation, I will also discuss a potential therapeutic agent called disaggregase that can modulate pathological phase transition of ANXA11.

Keywords: amyotrophic lateral sclerosis (ALS), RNA binding protein, FTD, TDP, ANXA11

S48-3

Therapeutic strategies of autologous MSC for ALS patients: current and future perspectives

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by selective and progressive loss of motor neurons. Disease progression leads to death within 3 to 5 years. The pathophysiological mechanisms of cell death (mostly of motor neurons) in ALS remain unclear. Still, recent studies using models of SOD1 mutant and ALS- associated genes including C9orf72, TDP43, FUS, TBK1, and ANXA11 revealed that diverse molecular mechanisms such as altered protein degradation, RNA dysregulation, oxidative stress, glutamate toxicity, mitochondrial dysfunction, altered immune-inflammation, and abnormal axonal transport are related with ALS. Previous ALS clinical trials based on single molecular targets suggest the importance of the integration of multiple molecular targets in the overall therapeutic strategy. Currently, there are no curative therapeutic agents for the treatment of ALS. Riluzole, edaravone, and Albrioza are the only approved drugs by the Food and Drug Administration with modest treatment effects on ALS progression. Numerous new strategies have been developed for the treatment of ALS. Previous failures of clinical trials of ALS have suggested novel therapeutic approaches, including stem cell and genetics-based strategies. Stem cells exert diverse actions, such as stimulating intrinsic cell replacement, neurogenesis, releasing diverse neurotrophic factors, and modulating immuno-inflammatory processes. Because of the diverse stem cell effects, stem cell therapy is an emerging alternative therapeutic or disease-modifying strategy for ALS. There are several types of stem cells utilized; embryonic stem cells, induced pluripotent stem cells, neural progenitor cells, and mesenchymal stem cells (MSC). In this review, we summarize recent advances in the treatments and the limitations of MSC technologies. We especially, focus on clinical trials of MSC therapies for ALS.

Keywords: Amyotrophic lateral sclerosis (ALS), mesenchymal stem cells, clinical trial, treatment

S48-4

New horizons of alzheimer's disease treatment strategy: Focusing on newly approved amyloid-based monoclonal antibodies

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Alzheimer's disease is a rapidly growing neurodegenerative disorder, driven by the increasing elderly population. The formation of amyloid beta (Aβ) deposits is recognized as a key mechanism in Alzheimer's disease. Based on these research findings, newly approved amyloid-based monoclonal antibodies are expected to play a pivotal role in the treatment strategy for Alzheimer's disease. In this session, I present an evaluation of the efficacy and safety of amyloid-based monoclonal antibodies, based on the latest research findings and clinical trial data. Additionally, we provide an in-depth understanding of the mechanism of action and therapeutic effects of these drugs. Drawing on recent clinical trial results targeting the newly approved amyloid-based monoclonal antibodies, we investigate their impact on symptom alleviation and disease progression in Alzheimer's disease. These research findings contribute to advancing our understanding of Alzheimer's disease treatment and developing novel therapeutic strategies. Furthermore, we discuss potential side effects and limitations of amyloid-based monoclonal antibody therapy. By examining ongoing and future research directions to overcome these side effects and limitations, we aim to explore new perspectives in the treatment strategy for Alzheimer's disease. Through this abstract, we present the new horizons of Alzheimer's disease treatment strategy, focusing on the newly approved amyloid-based monoclonal antibodies, thus shedding light on the advancements in Alzheimer's disease research and potential breakthroughs in treatment options.

Keywords: Alzheimer's disease, amyloid-based monoclonal antibodies, amyloid beta, treatment

S48-5

Targeting TDP-43 aggregation improves the phenotype of amyotrophic lateral sclerosis

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The large majority of ALS patients (>97%) harbor TDP-43 cytoplasmic aggregates regardless of etiology, highlighting the importance of understanding TDP-43 toxicity mechanisms. Thus reducing the cytosolic aggregation of TDP might be one of the major target to ameliorate the TDP-43 proteinopathy. So far, several approaches have attempted to lessen the TDP-43 aggregation using various modalities such as single-chain variable fragment (scFv) and proteolysis targeting chimera (PROTAC). In this talk, a novel approach to reduce the TDP-43 aggregation will be introduced. A novel nanoparticle can inhibit the fibrilization of the peptides. It also decreases stress granule (SG) formation *in vitro*. The beneficial effect of the nanoparticle was also observed in TDP-43 mouse models. Administration of the nanoparticle retarded the onset and survival of TDP-43^{A315T} and TDP-43^{WT} transgenic mice through enhancing motor neuron survival and reducing glial activation. The therapeutic efficacy of the nanoparticle was also extended to other type of ALS such as FUS and C9orf72 mutations. It reduced FUS-mediated SG formation and improved the neuropathic symptoms of C9orf72 mutant mice. Therefore, the nanoparticle exhibited potent therapeutic benefits to most of ALS patients via targeting TDP-43 aggregation or SG formation.

Keywords: Amyotrophic lateral sclerosis (ALS), TDP, proteinopathy, C9orf72

S49-1

A systematic review of the physiological effects of traditional regional diets targeting the prevention of cardiovascular disease

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Aim: Traditional regional diets are sustainable dietary patterns with a plethora of health benefits. The aim of the present systematic review was to pool all evidence on the physiologic effects of regional diets among adults at a high risk for developing cardiovascular disease (CVD).
Methods: Three databases were searched for randomized controlled trials (RCTs) implementing any regional dietary pattern, including the Mediterranean (MD), Japanese, Chinese, Persian, Mexican, Southern European Atlantic, new Nordic, or other, while examining cardiovascular risk factors among adults with elevated risk. Primary outcomes included anthropometric measures and secondary outcomes involved blood lipid levels, glucose metabolism (fasting plasma glucose and insulin, insulin resistance, HbA1c), inflammation (TNF1, IL-6, CRP, etc.), and other CVD markers (Flow Mediated Dilatation, ADMA, etc.).
Results: Twenty RCTs fulfilled the study's criteria and were included in the qualitative synthesis, with the majority implementing a MD. Adherence to most of the regional diets induced a reduction in body weight and improvement of anthropometric indices among participants. The majority of RCTs with blood pressure endpoints failed to induce a significant reduction in the intervention compared to the comparator arms, with the exception of few new Nordic and MD ones. Despite the interventions, inflammation markers remained unchanged except for CRP, which was lowered in the intervention groups of one new Nordic, the older Japanese and the Atlantic diet RCTs. With regard to blood lipids, regional diet interventions either failed to induce significant differences, or improved the lipid profile of participants adhering to the experimental regional diets. Finally, in the majority of included trials, no improvement was noted regarding glucose metabolism.
Conclusion: The reviewed body of evidence suggests that adherence to regional diets may reduce specific cardiovascular risk factors, with the MD appearing to confer more health-related benefits. Nonetheless, the body of evidence is still limited for most of the examined regional diets and a great heterogeneity is observed in the studies.

Keywords: cardiovascular risk factors, obesity, nutrition transition, body composition, lifestyle medicine

S49-2

Effects of long- and short-term exposure to the mediterranean diet on skin microvascular physiology

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The Mediterranean Diet (MD) is a collective term for the dietary patterns consumed by the people inhabiting the Mediterranean countries. It involves increased consumption of olive oil, vegetables, legumes, oily fish, cheese, nuts and moderate amounts of white meat and wine. MD appears to provide clinical benefits in reducing the risk for CVD, diabetes and cancer. Although the MD benefits are considered to be significant, there are certain aspects that need further clarification—for example, it is not known as of whether the improved physiological effects in skin microvascular health from the long-term adherence to the MD are greater to those observed following a short-term adherence (as someone would expect them to be) and if they are, to what extent. We have completed a series of studies, in the UK and in Greece, targeting non-clinical-but-sedentary adults, exploring: a) whether short-term adherence to the Mediterranean diet (MD) was associated with improved physiological function, and b) to assess the effects of long- vs. short-term MD adherence on the skin microvascular circulation, and quality of life. Our findings indicate that although short-term MD adherence can be effective in improving specific microvascular physiological properties and QoL domains, in specific non-clinical groups (e.g., people younger than 35 years of age), there is room for additional improvement, observed in long-term adherers, irrespective of age. Our findings are important in the design of future, MD-based, lifestyle interventions, with the advisable durations differing between target groups.

Keywords: microcirculation, Mediterranean diet, sedentary adults, long-term adherence, short-term adherence

S49-3

Contribution of traditional Japanese diet on cardiovascular health but not on musculoskeletal health - possible association of adiponectin

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How regional diets contribute to cardiovascular health is a complex question from pathophysiological point of view because of following reasons; diets contain multitude of nutrients, dietary behavior may largely vary not only among people but also within a single individual temporally, quantitively and qualitatively, physiological response to a given diet may be mediated not only by complex procedure of digestion which could be affected by homeostatic regulation but also microbiome and various environmental exposure. One of the effective ways to grab the potential causal relationship between diet and cardiovascular health is epidemiological approach with a prospective cohort design. Food frequency questionnaires are commonly used tool to collectively assess the dietary exposure. Outcome measures are incident cardiovascular diseases or acquisition of major cardiovascular risk factors such as obesity, elevated blood lipid levels and/or glucose levels, higher blood pressure levels, or inflammatory markers as surrogate measures. Involvement of potential mediators could be identified mainly by blood biomarkers through regression analyses. In this symposium we would like to show our previous studies in which we identified potential contribution of traditional Japanese diet on cardiovascular health possibly mediated by adiponectin(1,2). Interestingly, however, too high level of adiponectin may compromise musculoskeletal function (3,4), which may lead to shorter disability free life expectancy or higher incidence of fall related fracture (5,6). In addition, there is an advantage of examining the possible interaction and association of other lifestyle factors such as physical activity in the analyses. We will also present an emerging method of data analysis using machine learning to categorize diet and dietary behavior.

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Keywords: cardiovascular risk factor, musculoskeletal health, adiponectin, dietary pattern, cohort study

S49-4

Physiological effects of the new nordic diet on high cardiovascular disease-risk adults

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Background: The New Nordic diet (NND) is a novel diet designed to improve health that advocates the consumption of food ingredients typical of Nordic countries, such as winter vegetables and fruits, wholegrains, legumes, fish, and canola oil. Health benefits of traditional Nordic-type diets have become apparent: systematic reviews and meta-analyses of prospective cohort studies observe reductions in CVD incidence and mortality, CHD and stroke incidence, and reduced incidence of type II diabetes in adults with and without type 2 diabetes. Increasing interventional research explores the physiological effects of NND in individuals at risk of developing CVD, highlighting possible benefits for ameliorating cardiovascular health risk. This narrative summary examines emerging evidence for physiologic effects of the NND for individuals at risk of developing cardiovascular disease.
Methods: Eight RCTs and two pilot studies investigating effects of the ND were narratively reviewed for physiological effects related to CVD risk, including anthropometric data, blood pressure, fasting blood glucose / insulin, blood lipids, inflammatory markers, and microvascular assessments in individuals at increased risk of cardiovascular disease (≥ 55 yrs. and/or presence of at least one risk factor).
Results: Evidence from 6 RCTs and 2 pilot studies indicated that the NND can lead to weight loss, improved BMI, and body composition. Blood pressure improvements were observed in 6 studies, including 3 RCTs. Changes to blood lipids including reduced Total Cholesterol and improvements in LDL-C, LDL/HDL ratio, ApoB /$ApoA_1$, ApoB, $ApoA_1$ were observed in 7 studies, including 6 RCTs, although findings were mixed. Improvements in CRP and hsCRP were observed in two RCTs. Reductions in fasting insulin, HOMA-IR, Matsuda index were observed in 3 RCTs. Axon-mediated vasodilation and peak heart rate during submaximal exercise improvements were observed in older participants in one pilot study, suggesting possible microvascular benefits.
Conclusion: The NND appears promising for weight loss, anthropometric indices, and possess the potential to improve blood

lipids, with limited or unclear evidence for other risk factors currently. The NND shares nutritional characteristics with the Mediterranean Diet, which has robust evidence, however more work is needed to explore potential health benefits of the NND over longer intervention periods.

Keywords: Nordic diet, microvascular function, older adults

S50-1

The interactions between eosinophils and mast cells (the allergic effector unit) as the ultimate pro-inflammatory cross-talk?

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The recognized pivotal effector cells of allergic inflammation (AI) are the mast cells and the eosinophils. Mast cells, as activated by IgE-dependent mechanisms via allergens, are the starters while eosinophils infiltration and persistence in the inflamed tissue with the mast cells are the accepted features of the late stage and of the chronic outcome of allergy.
In the past we defined a pro-inflammatory cross-talk between mast cells and eosinophils that we named the Allergic Effector Unit (AEU). We found that mast cell/eosinophil interactions result in increased eosinophils chemotaxis, survival, degranulation, cytokine production and in increased mast cell survival, IgE-dependent and independent degranulation and cytokine production. These effects are mediated by both released mediators (soluble interactions) and by receptor/ligands binding (physical interactions). Prominent players of the activating "physical" AEU are the two activating receptors (ARs)/ligands CD48 and 2B4. Yet, at the same time we also described the presence and functional activity of two inhibitory receptors (IRs), i.e., CD300a and Siglec-7, on mast cells and on eosinophils that can indicate a possible anti-inflammatory or even pro-resolution activity of the cross-talking cells, within the AEU and in the allergic inflamed environment. We have identified in an allergic peritonitis model a pro-resolution pathway that is modulated by CD300a and the specialized pro-resolving lipid mediator (SPM) Lipoxin A_4. Most importantly, we recently found that IgE-activated mast cells produce the SPM Resolvin D_1 and could therefore orchestrate in cross-talk with other immune cells resolution in allergy. Moreover, both mast cell and eosinophil functions can be downregulated by SPMs.
Therefore, by analysis of the AEU *in vitro* and *in vivo* in mouse models of AI, and finally in *ex vivo* samples from allergic patients we have defined as potential new and better targets for immunopharmacological intervention in allergic diseases the strategy of blocking ARs, i.e., CD48, and/or by activating IRs, i.e., CD300a and Siglec-7, and by inducing a pro-resolution phenotype in mast cells. Therefore, agonistic and mast cell targeted anti-CD300a antibodies are an important tool for treatment of allergic diseases. Lastly, we found that mast cells can produce the pro-resolving lipid mediator Resolvin D1 and hence have the potential of not only initiating allergy but also resolving it.
Thus, our strategy is to treat allergic diseases by inhibiting activation and/or by activating inhibition mostly of mast cells and the AEU, and by bolstering the pro-resolution properties of the mast cells. Translationally this strategy will have to take into consideration the complexity of allergic patient endotypes.

Keywords: eosinophils, mast cells, allergic effector unit, inflammation, resolution

S50-2

Renal tubular urate transporters and hyperuricemia

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Uric acid is a poorly soluble substance with a molecular weight of 168 and a pKa value of 5.75, and exists as an organic acid in the body. In most mammals, uric acid is an intermediate metabolite of purines, but in humans and primates (chimpanzees, gorillas, orangutans, gibbons, etc.), uric acid is the final metabolite of purines since uric acid oxidase (uricase) in the liver is deleted due to mutations. Two-thirds of uric acid produced in the body mainly by the liver is excreted by the kidneys. However, the kidneys reabsorb uric acid, and only about 10% of the glomerular filtration rate is excreted in the urine. In other words, 90% is reabsorbed. It is known that the accumulation of uric acid in the body causes hyperuricemia, which can lead to gouty arthritis, urinary tract stones, kidney damage (gouty kidney), high blood pressure, cardiovascular disease, and metabolic syndrome. Although the accumulation of uric acid, which is considered to be harmful, is disadvantageous for living organisms, humans exhibit higher blood uric acid levels than other mammals due to the existence of a reabsorption mechanism in the kidneys. Interestingly, while uric acid exhibits an oxidizing effect in cells and tissues, it is also known that it exhibits an antioxidant effect within blood vessels, and is thought to have a dual nature. The blood concentration of uric acid is mainly determined by uric acid excretion in the kidneys, and uric acid transporters in the kidneys are deeply involved. In this lecture, I would like to talk about the dual nature of uric acid, the mechanism by which the kidneys control serum uric acid levels, and the mechanism by which this mechanism breaks down, causing hypouricemia and hyperuricemia.

Keywords: urate, kidney, reabsorption, transporters, uricosurics

S50-3

Role of CHIP ubiquitin ligase in hepatic steatosis

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TXNIP is a critical regulator of glucose homeostasis, fatty acid synthesis, and cholesterol accumulation in the liver, and it has been reported that metabolic diseases, such as obesity, atherosclerosis, hyperlipidemia, type 2 diabetes, and nonalcoholic fatty liver disease (NAFLD), are associated with endoplasmic reticulum (ER) stress. Because CHIP, an E3 ligase, was known to be involved in regulating tissue injury and inflammation in liver, its role in regulating ER stress-induced NAFLD was investigated in two experimental NAFLD models, a tunicamycin (TM)-induced and other diet-induced NAFLD mice models. In the TM-induced NAFLD model, intraperitoneal injection of TM induced liver steatosis in both $CHIP^{+/+}$ and $CHIP^{+/-}$ mice, but it was severely exacerbated in $CHIP^{+/-}$ mice compared to $CHIP^{+/+}$ mice. Key regulators of ER stress and de novo lipogenesis were also enhanced in the livers of TM-inoculated $CHIP^{+/-}$ mice. Furthermore, in the diet-induced NAFLD models, $CHIP^{+/-}$ mice developed severely impaired glucose tolerance, insulin resistance and hepatic steatosis compared to $CHIP^{+/+}$ mice. Interestingly, CHIP promoted ubiquitin-dependent degradation of TXNIP in vitro, and inhibition of TXNIP was further found to alleviate the inflammation and ER stress responses increased by CHIP inhibition. In addition, the

expression of TXNIP was increased in mice deficient in CHIP in the TM- and diet-induced models. These findings suggest that CHIP modulates ER stress and inflammatory responses by inhibiting TXNIP, and that CHIP protects against TM- or HF-HS diet-induced NAFLD and serves as a potential therapeutic means for treating liver diseases.

Keywords: CHIP, endoplasmic reticulum, metabolic disease, NAFLD, TXNIP

S50-4

IRF3/IP-10 axis as a new target for atopic dermatitis

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Background: Despite the relevance of IFN-γ-inducible protein 10 (IP-10; CXCL10), which is well-known in innate Th2 immune responses, its role in atopic dermatitis (AD), a representative Th2 immune disorder, is still unclear.
Methods: The functional relevance of IP-10 in AD was evaluated using house dust mite (HDM)-sensitized human AD tissue and a *Dermatophagoides farinae* extract (DFE)-induced AD mouse model. Further, the functional mechanism of IP-10 was analyzed using several T lymphocytes.
Results: IP-10 expression was concentrated in the epithelium of the lesions, which was dependent on the HDM-sensitization level. In particular, mite allergen stimulation altered the expression of IP-10 according to the differentiation stage of keratinocytes. In the AD mouse model, IP-10-knockout (KO) compared to wild-type (WT) mice showed notably reduced representative AD phenotypes, including Th1 and Th2 responses. In contrast, IP-10 subcutaneous injection into WT skin lesions deteriorated the pathophysiological changes. Particularly, increased Th2 responses and Th2 cell populations were observed within the auricular lymph nodes. These results were also observed in KO AD mice injected with IP-10, inducing amplified Th2 responses compared to PBS-injected AD mice. IP-10 treatment in naïve T cells *in vitro* promoted the differentiation to Th2 cells. In addition, IP-10 in Th2 cells directly increased IL-4 expression.
Conclusion: We conclude that IP-10 is specifically induced in DFE-stimulated skin keratinocytes and acts as an essential mediator that exacerbates allergic skin inflammation by activating Th2 cells.

Keywords: atopic dermatitis, house dust mite, IFN-Î³-inducible protein 10, interleukin-4, Th2 inflammation

S51-1

Regulation of fat metabolism by exercise-induced metabolic intermediates

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Fat browning contributes to energy consumption and may have metabolic benefits against obesity; however the potential roles of lactate and beta-hydroxybutyrate (β-HB) in fat browning remain unclear. We investigated the roles of a single bout of aerobic exercise that increases lactate and β-HB levels in the fasted state on the regulation of fat browning in rats and humans. In addition, we evaluated the effects of chronic hyperketonemia induced by ketogenic diet and/or endurance exercise on the fat browning in rats. Furthermore, we investigated the mechanisms of the fat browning effects of β-HB in adipocytes.
Regardless of fasting, single bout of exercise increases the concentration of lactate and β-HB in rats, but the exercise in the fasted state increases the β-HB level more significantly in rats and humans. Exercise in the fasted state activated fat browning (AMPK/SirT1/PGC1a pathway and PRDM16) and thermogenic factor (UCP1) in white fat of rats. In rats and humans, exercise in the fasted state increased the blood levels of fat browning-related adipomyokines. In particular, compared to F-OBLA, F-HIIE (high-intensity interval exercise) more efficiently increases free fatty acid (FFA) as well as blood levels of fat browning adipomyokines in humans, which was correlated with blood levels of lactate and β-HB. In rats performed exercise with different intensity, the higher plasma lactate and β-HB levels, and higher expression of p-AMPK, UCP1 and PRDM16 in white adipose tissue of HIIE group than those of moderate intensity group were observed. In chronic intervention of ketogenic diet and/or exercise, intracellular β-HB activated fat browning programs and thermogenic factors in adipocytes, which was further confirmed in white adipose tissues (WATs) of rats. Additionally, β-HB induces lipolysis and the expression of fat browning-related adipomyokines such as FGF21 and irisin in adipocytes. In rats subjected to an 8-week intervention of aerobic exercise training and/or a ketogenic diet, we observed a significant reduction in body weight and blood lipid levels, accompanied by the activation of WAT browning. Furthermore, we found a negative correlation between the levels of β-HB after intervention and blood lipid levels. Notably, a combined intervention of exercise and ketogenic diet showed additive effects on fat browning.
In conclusion, a single bout of aerobic exercise in the fasted state significantly induced fat browning-related pathways, FFA and adipomyokines, particularly F-HIIE in human. The fat browning effects of β-HB were reproduced in a condition of chronic hyperketonemia in rats. Although further evidences for supporting our results are required in humans, aerobic exercise in the fasted state with high intensity that increase lactate and β-HB, or chronic intervention that increase the β-HB levels may be a modality of fat browning.

Keywords: beta-hydroxybutyrate, fat browning, obesity, thermogenesis, lactate

S51-2

The role of cereblon in exercise-induced animal and human models

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Background: Cereblon (CRBN) serves as a substrate receptor of the E3 ubiquitin ligase complex and plays a critical role in regulating AMPK activity. Exercise is known to be a key modulator of AMPK. However, the precise cellular mechanisms governing CRBN regulation during exercise training remain unclear.
Methods: In this study, we investigated the role of CRBN during exercise in both animal and human models. We conducted aerobic exercise experiments in STZ-induced animal models and examined its impact on CRBN and AMPK levels in skeletal muscle. Additionally, we evaluated the effects of a 12-week aerobic exercise regimen on CRBN serum levels in pre-diabetic patients and its relationship with aerobic exercise capacity. Resistance exercise was also studied for its impact on CRBN serum levels. Furthermore, we conducted experiments involving

moderate acute exercise in college students and explored its influence on CRBN serum levels.
Results: Our findings indicate that aerobic exercise leads to a decrease in CRBN levels and an increase in AMPK levels in the skeletal muscle of STZ-induced animal models, resulting in improved glucose metabolism. Additionally, a 12-week aerobic exercise program was associated with decreased CRBN serum levels in pre-diabetic patients. Notably, the reduction in CRBN serum levels was negatively correlated with aerobic exercise capacity in these patients. In contrast, resistance exercise did not induce changes in CRBN serum levels. Furthermore, moderate acute exercise resulted in decreased CRBN serum levels in college students, while acute high-intensity exercise exhibited a trend towards increased CRBN serum levels.
Conclusion: Our study suggests that aerobic exercise has a significant impact on reducing CRBN levels in both serum and skeletal muscle. Moreover, our results indicate that different types of exercise may regulate CRBN levels, potentially making CRBN a target for exercise-induced metabolic alterations. These findings contribute to a better understanding of the intricate cellular mechanisms involved in exercise-induced adaptations.

Keywords: exercise, cereblon, skeletal muscle, pre-diabetes, heart

S51-3

Can exercise intervention reverse the impairment of endothelial TRPV4 channel function in obesity?

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Background: Obesity-mediated cardiovascular comorbidities result from endothelial dysfunction. Although it is well known that endothelial Ca^{2+} signaling regulates endothelium-dependent vasodilation in small arteries, it still remains unclear whether endothelial Ca^{2+} signaling mechanisms are compromised in obesity. The unitary Ca^{2+} influx through TRPV4 (transient receptor potential vanilloid 4) channels, termed TRPV4 Ca^{2+} sparklets, induces vasodilation through the activation of Ca^{2+}-sensitive K^+ (IK and SK) channels. A crucial regulator of TRPV4 sparklet activity is the kinase anchoring protein 150 (AKAP150), which may bind protein kinases A and C (PKA and PKC).
Methods: In this study, we investigated whether obesity affects the control of endothelial Ca^{2+} signaling via AKAP150-TRPV4 regulation. It was found that systolic blood pressure was increased, and endothelium-dependent vasodilation of third-order mesenteric arteries (MAs) was impaired in the novel endothelium-specific AKAP150-/- mice. In MAs from C57BL/6J mice, TRPV4 channel activity and vascular function were assessed after 12 weeks of a normal (10% kcal) or high fat (60% kcal) diet.
Results: While the vasodilatory responses to IK/SK channel activators were not changed in obese mice, vasodilation was markedly attenuated in the presence of selective TRPV4 channel agonist (GSK1016790A, GSK101, 3~30nM). It was suggested that obesity disrupts TRPV4 channel activity but not downstream signaling or endothelial-smooth muscle communication. TRPV4 sparklet activity in response to 10 nM GSK101 was reduced by 2-fold in fluo-4-loaded *en face* MAs from obese mice. TRPV4 expression at the mRNA and protein levels was unaffected by obesity, indicating that channel regulation is likely to be disturbed in other mechanisms rather than channel expression. Although the mechanisms for peroxynitrite (PN)-induced endothelial dysfunction are still unknown, PN is associated with endothelial dysfunction in obesity. In obese mice, PN scavenger (uric acid) restored diminished TRPV4 sparklet activity and vasodilation. Therefore, we proposed that PN reduces the ability of AKAP150-TRPV4 complex to regulate endothelial Ca^{2+} signaling in obesity. Exogenous PN significantly reduced TRPV4 activity and vasodilation by three times in normal MAs, which was prevented by pretreatment with uric acid. Furthermore, obese MAs displayed higher levels of 3-nitrotyrosine (protein nitration product of PN), primarily at myoendothelial projections, where the majority of TRPV4 sparklet activity occurs and AKAP150 is concentrated.
Conclusion: Obesity-induced increase in PN may cause nitrotyrosin formation on AKAP150, which inhibit TRPV4 sparklet activity and vasodilation.

Keywords: calcium signaling, endothelium, ion channel, nitric oxide, peroxynitrite

S51-4

Novel direction for potential restorative effects of bone metabolism with aerobic and resistance combined exercise in ovariectomized (OVX)-induced osteoporotic rats

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Background: Post-menopausal leads to significant bone loss and is associated with an elevated risk of osteoporosis. Multimodal exercise training is recommended to slow menopausal-associated bone loss. Among the effects of exercise on bones, a recent emerging topic is the role of bone-associated skeletal nerves, and it has been reported that aerobic exercise training increases the nerve fiber density of the periosteum. Yet, so far, the effect of combined exercise on skeletal nerves and ultimately on bone volume have not been studied. We sought to determine the potential restorative effect of combined exercise on trabecular bone metabolism and skeletal nerve density of the tibia following ovariectomy (OVX) in rats.
Methods: Twenty-eight female Fisher-344 rats were randomly assigned 8 weeks of either (a) Sham non-exercise (SN), (b) Sham combined exercise (SE), (c) Ovariectomy non-exercise (ON), or (d) Ovariectomy combined exercise (OE) groups (n=7). A combined exercise consisting of tower climbing resistance and treadmill running aerobic exercise was loaded for 8 weeks to compare and analyze the changes in the proximal tibia trabecular bone microarchitecture by micro-computed tomography. Immunofluorescent imaging was used to analyze bone-associated skeletal nerve density.
Results: Ovariectomy induced a significant ($P<0.01$) decrease in serum estrogen level compared to the Sham operation group. Estrogen deficiency led to an increase in body weight but decreased bone volume following OVX. Trabecular bone volume (BV, mm^3) and fractional bone volume (BV/TV, %) were significantly higher in both sham and ovariectomized animals ($P<0.05$) following combined exercise compared to each group that did not perform the exercise. Accordingly, trabecular number (Tb.N, 1/mm) was significantly increased in SE and tended to be higher ($P<0.10$) in OE than in non-exercise groups, while trabecular separation (Tb.Sp, μm) was reduced with combined exercise in SE vs. SN. Interestingly, OVX significantly reduced bone-associated skeletal nerve fibers (SN, 859.4±310.2 vs. ON, 195.7±43.1, $p<0.01$), but combined exercise training partially restored the nerve fiber density approximately by 135% (ON vs. OE, $p<0.01$). Combined exercise alone showed a positive influence on skeletal nerve fibers, whereby greater nerve density was observed in SE (SN, 859.4±310.2 vs. SE, 1407.8±396.9, $p<0.01$). Correlation analyses revealed a significant association between BV/TV and skeletal nerve fibers in all groups except for SE (SN; $R^2=0.81$, $p<0.01$, ON; $R^2=0.45$, $p<0.05$, SE; $R^2=0.31$, $p=0.09$, OE; $R^2=0.78$, $p<0.01$). Bone mineral density (BMD) was not different between non-exercise

and combined exercise groups.
Conclusion: 8-weeks of combined exercise is a positive exercise regimen on tibial trabecular bone microarchitecture by stimulating bone-associated skeletal nerve fibers. Combined exercise training may have a dual role as a direct stimulator of bone remodeling and skeletal nerve regeneration.

Keywords: ovariectomy, trabecular bone microarchitecture, bone-associated skeletal nerve fibers, combined exercise, mRNA expression

S51-5

Decoding exercise at molecular levels and health

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Exercise has beneficial effects on several organs. A biochemical understanding of exercise has been considered a novel pharmaceutical strategy to find molecules to deliver exercise effects. These effects are often mediated by myokines, muscle-secreted factors for tissue crosstalk. Irisin is a myokine induced by exercise in skeletal muscle. Irisin is a polypeptide of 12kDa that is cleaved from a type I membrane protein called FNDC5. FNDC5 is expressed mainly in skeletal muscle, heart, and brain. FNDC5 mRNA increases in adult human muscles with several forms of endurance exercise. Advanced Tandem Mass Spectrometry has demonstrated that human irisin circulates at "hormone-like" levels and increases as a consequence of endurance exercise. Since its discovery in 2012, irisin has been shown to affect bone, fat, and brain. In many cases, irisin's effects are reminiscent of those derived from physical exercise, including improved cognition in mice. Here, we identified the major receptor for irisin as the αV integrin family and its cofactor as cluster of differentiation 81 (CD81) with quantitative proteomics using mass spectrometry. Irisin treatment increased phosphorylation of focal adhesion kinase (FAK), and genetic deletion of CD81 or treatment of integrin αV inhibitors blunted the signaling. Genetic deletion of integrin β1 or integrin β5, dimer partners of integrin αV, prevented irisin-induced FAK phosphorylation. Irisin treatment delivered endurance exercise effects including bone remodeling and fat thermogenesis via the integrin αV family. Genetic deletion of CD81 blocked irisin-induced thermogenic fat cell proliferation *in vitro* and worsened metabolic phenotypes upon high-fat diet challenge. Overall, this study suggests that irisin can be utilized for therapeutic approaches to find cures for several diseases which can be relieved by endurance exercise.

Keywords: exercise, myokine, irisin, integrin, metabolism

S29-1

Oligodendrocyte precursor cells shape inhibition in medial prefrontal cortex

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Cortical inhibition is performed by interneurons releasing GABA that acts on ionotropic $GABA_A$ and metabotropic $GABA_B$ receptors. Therefore, interneuron density, firing activity and myelination are pivotal determinants of correct inhibitory circuits in the central nervous system (CNS). One of the regulatory processes that defines the cortical interneuron density is the programmed cell death at the first two postnatal weeks. The apoptosis of interneurons increases drastically after postnatal day 5 and reaches its peak at p7. Of note, the synapses between oligodendrocyte precursor cells (OPCs) and interneurons are formed about postnatal day (p) 4-5, suggesting OPC-interneuron connection might contribute to the interneuron elimination. By inducing conditional ablation of $GABA_BR$ subunit 1 *gabbr1* in OPCs, we identified GABA/tumor necrosis factor (TNF)-like cytokine (TNFSF12 or TWEAK)-mediated bidirectional communication pathway between interneurons and OPCs that determines the density and function of interneurons in the developing medial prefrontal cortex. GABA, activating $GABA_BRs$ of OPCs (OPC-$GABA_BRs$), triggers OPCs to release TWEAK (TNF-like weak inducer of apoptosis), which in turn specifically induces interneuron apoptosis. When the GABAergic signaling to OPCs were genetically interrupted, an increased density of PV^+ interneurons was found. Despite of a higher number of surviving interneurons, the inhibitory tone in the mutant mouse brain was significantly suppressed. In addition, these interneurons were less myelinated. Consequently, the mutant mice exhibited strong changes of cortical network activities and impaired social cognitive behavior. Taken together, our study demonstrates that $GABA_BRs$ of OPCs are crucial elements in finetuning distinct brain functions.

Keywords: oligodendrocyte precursor cells, interneuron, GABA, TWEAK, cognition

S29-2

Metaplasticity augmentation by acid glia in cerebellar motor learning

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Plastic change in the efficiency of signal transmission between neurons underlies the formation of memory and adaptive behavior. Short-term memory is generated by rapid potentiation or depression of synaptic transmission. Engravement of long-term memory requires physical formation or deconstruction of the synaptic structure constituting the neuronal circuit. Traditionally, these two memory formation processes were considered to occur serially; i.e. synapses that rapidly changed their signal transmission efficiency will subsequently be structurally stabilized or pruned. However, our study has shown that short and long-term memory are formed in parallel. Genetic deletion of glutamate-releasing anion channels in glial cells or optogenetic manipulation of glial activity specifically impaired or augmented the online learning during the training period while the offline learning that developed upon the rest period remained intact. Glia senses the synaptic activity in the surroundings and releases glutamate from their cytosol via anion channels, which amplify synaptic signals. This glial amplification mechanism is required for efficient adaptive control of motor behavior. We also found that cerebellar Bergmann glial cells 'eat' their neighboring neuronal elements within healthy living brain tissue. This Bergmann glial engulfing of synapses was enhanced upon motor learning in mice's cerebellum. Moreover, pharmacological blocking of this engulfment inhibited synaptic structural changes, resulting in part of the offline learning and memory process being lost. These results show that online and offline learnings are parallel processes and both of the learnings are differentially affected by the glial activity.

Keywords: astrocyte, cerebellum, Bergmann glia, motor learning, plasticity

S29-3

Microglia modulate general anesthesia through $P2Y_{12}$ receptor signaling

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General anesthesia (GA) is an unconscious state produced by anesthetic drugs, which act on neurons to cause overall suppression of neuronal activity in the brain. Recent studies have revealed that GA also substantially enhanced the dynamics of microglia, the primary brain immune cells, with increased processes motility and territory surveillance. However, whether microglia are actively involved in GA modulation remains unknown. Here, we report a previously unrecognized role for microglia engaging in multiple GA processes. We unexpectedly found that microglia ablation reduced the sensitivity of mice to anesthetics and substantially shortened duration of loss of righting reflex (LORR) or unconsciousness induced by multiple anesthetics, thereby promoting earlier emergence from GA. Microglia repopulation restored the regular anesthetic recovery, and chemogenetic activation of microglia prolonged the duration of LORR. In addition, anesthesia-accompanying analgesia and hypothermia were also attenuated after microglia depletion. Single-cell RNA sequencing analyses showed that anesthesia prominently affected the transcriptional levels of chemotaxis and migration-related genes in microglia. By pharmacologically targeting different motility pathways, we found that blocking of $P2Y_{12}$ receptor ($P2Y_{12}R$) reduced the duration of LORR of mice. Moreover, genetic ablation of $P2Y_{12}R$ in microglia also promoted quicker recovery of mice from anesthesia, verifying the importance of microglial $P2Y_{12}R$ in anesthetic regulation. Our work presents the first evidence that microglia actively participate in multiple processes of GA through $P2Y_{12}R$-mediated signaling and expands the non-immune roles of microglia in the brain.

Keywords: microglia, general anesthesia, $P2RY_{12}$

S29-4

Quantitative proteome analysis to discover myelin proteins relevant for a healthy nervous system

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The functional capabilities of the nervous system decline in neurodegenerative disorders and with normal aging. It is thus crucial to understand how glial cells support axons and thereby enable normal nervous system functions. My team utilizes proteomic approaches to identify myelin proteins involved in the functions of myelinating cells for shaping and preserving myelinated axons. This is exemplified by the recent identification of CMTM5 (*chemokine-like factor-like MARVEL transmembrane-domain containing protein 5*) as a CNS myelin protein. Indeed, upon gene targeting we find that CMTM5 is required to prevent the progressive degeneration of CNS axons. When studying human myelin disorders in mouse models, and considering that humans and mice evolutionarily diverged approximately 85 million years ago, it is critical to know to what extent their myelin protein composition is similar. By quantitative proteomics of myelin purified from human white matter we find that the abundance of structural myelin proteins (PLP, MBP, CNP, SEPTIN8) correlates well with that in C57Bl/6N-mice. However, multiple other proteins were identified exclusively or predominantly in human or mouse myelin. This is exemplified by peripheral myelin protein-2 (PMP2), which was specific to human CNS myelin, while tetraspanin-2 (TSPAN2) was confined to mouse myelin. Assessing published scRNA-seq-datasets, human and mouse oligodendrocytes display well-correlating transcriptome profiles but divergent expression of distinct genes including *Pmp2* and *Tspan2*. A search interface is accessible via www.mpinat.mpg.de/myelin. Our results indicate that quantitative myelin proteome analysis enables to assess the heterogeneity of myelin composition across diverse conditions, and the functions of myelinating glia for a healthy nervous system.

Key publications

1. Gargareta VI et al. (2022) Conservation and divergence of myelin proteome and oligodendrocyte transcriptome profiles between humans and mice. eLIFE 11:e77019
2. Buscham TJ et al. (2022) Progressive axonopathy when oligodendrocytes lack the myelin protein CMTM5. eLIFE 11:e75523
3. Eichel MA et al. (2020) CMTM6 expressed on the adaxonal Schwann cell surface restricts axonal diameters in peripheral nerves. Nature Communications 11:4514
4. Siems SB et al. (2020) Proteome profile of peripheral myelin in healthy mice and in a neuropathy model. eLIFE 9:e51406

Keywords: glial cells, axon, myelin, nerve conduction, proteomics

S53-1

Development of novel strategies for cell-based cardiac repair/regeneration

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Ischemic heart disease (IHD) is the one of the major causes of death worldwide. Particularly, in the case of myocardial infarction (MI), patients ultimately advance to heart failure and die within short period from the onset of symptoms. Since the adult human heart has limited regenerative capacity, the infarcted areas by MI are rarely regenerated and are easily converted to non-contractile fibrotic scar, which lead eventual heart failure. Despite its high clinical significance, currently available therapeutic options including surgical and pharmacological interventions for treating heart failure are not very efficient as these strategies cannot directly repair the damaged hearts, rather only delay the progression of this detrimental disease. Therefore, significant efforts has been made in order to develop alternative strategies that can efficiently repair the failing heart. Recently, cell-based cardiac regeneration therapy has emerged as a promising therapeutic option. Particularly, cardiomyocytes derived from human pluripotent stem cells (hPSCs) including both embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) are regarded as one of the most promising sources for multiple applications such as development of new treatments for heart diseases, establishment of platforms for drug discovery and predictive toxicology, creation of *in vitro* models of human disease, and cardiac tissue engineering. While cardiomyocytes derived from hPSC (hPSC-CMs) are attractive sources for the use of heart repair and many other applications, numerous hurdles stand in the way of their clinical use. Their applicability is significantly limited by a number of major reasons such as i) low yields, ii) heterogeneity of differentiated hPSC-CMs, iii) immaturity of hPSC-CMs, and v) lack of optimal methods to deliver hPSC-CMs into the hearts. In my talk, I will discuss some of our previous studies and on-going studies to solve the current limitations in the use of hPSC-CMs for cardiac repair.

Keywords: heart disease, pluripotent stem cells, cardiomyocytes, endothelial cells

S53-2

Partial *in vivo* reprogramming enables injury-free intestinal regeneration via autonomous Ptgs1 induction

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Tissue regeneration after injury involves the dedifferentiation of somatic cells, a natural adaptive reprogramming that leads to the emergence of injury-responsive cells with fetal-like characteristics. However, there is no direct evidence that adaptive reprogramming involves a shared molecular mechanism with direct cellular reprogramming. Here, we induced dedifferentiation of intestinal epithelial cells using OSKM (Oct4, Sox2, Klf4, and c-Myc) *in vivo*. The OSKM-induced forced dedifferentiation showed similar molecular features of intestinal regeneration, including a transition from homeostatic cell types to injury-responsive-like cell types. These injury-responsive-like cells, sharing gene signatures of revival stem cells and atrophy-induced villus epithelial cells, actively assisted tissue regeneration following damage. In contrast to normal intestinal regeneration involving *Ptgs2* induction, the OSKM promotes autonomous production of prostaglandin E2 via epithelial *Ptgs1* expression. These results indicate prostaglandin synthesis is a common mechanism for intestinal regeneration, but involves a different enzyme when partial reprogramming is applied to the intestinal epithelium.

Keywords: reprogramming, regeneration, injury, dedifferentiation, Ptgs1

S53-3

Electrochemical detection of cellular metabolism and its application for stem cell-based drug screening

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Recently, stem cells have emerged as a promising source to generate various types of cells in vitro that are useful for toxicity assessment and drug screening. Organoids, multicellular tissue-mimicking constructs generated from stem cells, have shown immense potential to overcome the gap between in vitro testing and clinical trials. However, organoid generation from stem cells partially follows the developmental process of a complex multicellular organism and thus generally shows extreme variations in several parameters, including cell type, size, shape, and structure. To address such issues, here, we report a new tool that enables label-free and non-destructive assessment of the maturation level of kidney organoids derived from induced pluripotent stem cells (iPSCs). To reduce variations in an organoid generation that might be occurred due to the difference in initial cell number before inducing the differentiation, gold nanostructure-modified transparent indium tin oxide (ITO) was fabricated and further coated with Matrigel at specific concentrations. Thereafter, the correlation between the electrochemical signals and iPSC numbers was achieved to unify the initial cell numbers prior to the differentiation based on the electrochemical signal intensities. Interestingly, we found that two distinct electrochemical signals appeared at different potentials based on the cell types in the organoids. Specifically, the electrical signal that appeared at 0 V (vs. Ag/AgCl) corresponds to the off-target cell numbers while the same signals at 0.2 V (vs. Ag/AgCl) were found to be matched with the mRNA expression levels of CDH16, a proximal tubule early transcription factor. The peaks at 0.2 V completely disappeared on the kidney organoids that failed to show kidney-specific markers, including PECAM1 and NPHS1, indicating that these peaks are specific to the maturation levels of kidney organoids. We further found that the electrochemical signals of living cells originate from intracellular metabolic pathways that generate adenosine triphosphate (ATP). Based on this discovery, we demonstrated that the cellular signals detected by differential pulse voltammetry (DPV) can be rapidly amplified with a developed medium containing metabolic activator cocktails (MACs). The electrochemical approach combined with MAC treatment shows a remarkable performance to detect the effects of the anticancer drug CPI-613 on cervical cancer both at a low drug concentration and an extremely short treatment time (1 hour). Furthermore, the senescence of mesenchymal stem cells could also be sensitively quantified using the DPV+MAC method even at a low passage number (P6). Taken together, we can conclude that our new type of electrochemical platform is highly promising for label-free, non-destructive, and real-time monitoring of the viability of three-dimensional cellular constructs under various drug treatment conditions.

Keywords: stem cells, organoids, electrochemical detection, cellular metabolism, drug screening

S53-4

Disease modeling using patient-specific induced pluripotent stem cells

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Precision medicine is an emerging approach showing immense promise for disease prevention and treatment by considering the unique interplay of genes, environment, and lifestyle in each individual. A pivotal development in this context is the generation of human induced pluripotent stem cells (hiPSCs) sourced from patients. This not only bypasses ethical dilemmas tied to human embryonic stem cells but also sparks a revolution in the realm of human disease study. The potential is vast, as these hiPSC platforms could serve as transformative tools for preclinical trials, personalized clinical diagnoses, and tailored drug interventions. Here, I will present diverse hiPSC platforms and their applications, including unraveling precise disease mechanisms, accelerating drug exploration and assessment, establishing a "clinical trial in a dish" framework, and implementing precision medicine for patient care.

Keywords: induced pluripotent stem cells, disease modeling, drug screening

S54-1

Drug evaluation using human pluripotent stem cell-derived cardiac cell model

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Cardiac safety evaluation is one of the important factors in new drug development, and it requires good correlation between preclinical study

and clinical trial. However, animal models are used in the preclinical studies of drug development have limitations in predicting human responses due to interspecies differences. Therefore, the demand of new alternative approaches including human cell-based drug evaluation assays that can reflect human biology has been increasing globally. With recent advances in the field of stem cells research, human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes (CMs) have well developed to mimic human cardiac electrophysiology and broadly used in the evaluation of cardiac safety against the developing investigational drugs. For this reason, the ICH guideline was updated to be use hiPSC-derived CMs for cardiac safety evaluation in 2022. NEXEL produces highly functional hiPSC-derived CMs (Cardiosight®-S) that validated for the new test method in the updated guideline. Because the hiPSC-derived CMs including Cardiosight®-S shows actual human heartbeat and excellent electrophysiological characteristics, the cardiac safety evaluation method using hiPSC-derived CMs has been accepted in the global pharmaceutical industry and regulatory institute including US FDA. Furthermore, the hiPSC-derived CMs not only provide accurate prediction of cardiac toxicity through multi-ion channel analysis, but also provides the great accessibility by high throughput capability. Therefore, the use of hiPSC-derived CMs in the new drug development could a new paradigm under the updated ICH guidelines.

Keywords: iPSC, iPSC-derived cardiomyocyte, drug development, cardiotoxicity evaluation, cardiac safety assessment

S54-2

Chronic and acute drug-induced cardiotoxicity assessment using in vitro human iPSC-cardiomyocytes

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The Comprehensive in-vitro Proarrhythmia Assay (CiPA) paradigm was introduced to provide a more complete assessment of proarrhythmic risk by evaluating and implementing currently available high throughput methods. An important part of this is the electrophysiological evaluation of hERG, and also other cardiac channels including NaV1.5 and CaV1.2. The Q&A draft from August 2021 describes how nonclinical assays such as patch clamp can be used for integrated risk assessment prior to first-in-human studies, and in later stages of clinical development. Taking into account the newly proposed ICH/S7B guidelines for cardiac safety, we characterized commercially available human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) to complement the assays available for cardiac safety testing. Since the ICHS7 guidelines do not address chronic treatment-induced changes in blood pressure, heart rate and/or repolarization, we assessed the effects of acute and prolonged exposure of reference agents known to cause functional changes in the heart, using in vitro tools employing hiPSC-CMs. That includes Anthracyclines, tyrosine kinase inhibitors and trafficking inhibitors that have been linked to changes in cardiac function over prolonged exposure in patients. We further combined automated patch clamp recordings with a contractility monitoring technology, focusing on a mature cardiac phenotype. Here we studied the effects of calcium, sodium and hERG channel modulators at room temperature and at 37°C. Using hiPSC-CMs we show that late current (INa- Late), recorded during the ramp phase of the CiPA voltage protocol, was enhanced by ATX-II and blocked by ranolazine. Also mature physiological responses of hiPSC-CMs treated with positive inotropic substances such as L-type calcium channel agonist S-Bay K8644, beta- adrenergic agonist isoproterenol and cardiac myosin activator omecamtiv mecarbil are shown. Our data demonstrate that cardiac ion channel pharmacology can be successfully recorded using hiPSC-CMs in various platforms, providing a reliable tool for cardiac safety screening and the study of cardiac ion channel diseases in a model system closer to in vivo physiology than heterologous expression systems.

Keywords: CiPA, chronic, cardiac safety, contractility, electrophysiology

S54-3

High-throughput assessment of cardiac safety via calcium and voltage imaging of 3D engineered heart tissues and 2D cell cultures using a novel instrumentation platform

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Human induced pluripotent stem cell (hiPSC)-based assays are a promising approach for drug discovery but have shortcomings accurately modeling human physiology. For example, hiPSC-Cardiomyocytes (CMs) cultured with traditional methods mis-classify several cardiotoxic compounds. One likely reason is cellular maturity; most 2D culture techniques on flat surfaces result in fetal phenotypes delivering inaccurate results to compound exposure. More physiologically relevant systems, such as 3D Engineered Muscle Tissues (EMTs), have demonstrated more mature phenotypes, however the complexity and cost of 3D platforms limits their scalability. Adherent cell cultures grown on a nanostructured surface have shown improved maturity over flat 2D substrates and provide a useful intermediate for high throughput screening. Here, we report on the design, fabrication, and validation of a novel platform that uses 1) facile and scalable approaches to generate mature 2D and 3D models, and 2) parallel fluorescent measurements of Ca^{2+} and voltage from 3D engineered tissues and 2D cell cultures. Tissues and adherent cells were labeled with Cal-520 or FluoVolt. Groups of 4 tissues (64 wells) in 24 (384) well plates were imaged at 250 Hz to capture videos of full plates in 6 regions of interest. We investigated the differential effect of previously characterized drugs on EMTs and adherent cells cultured on standard tissue culture or nanostructured surfaces. Ca^{2+} and voltage signals had SNR greater than 40 and 10, respectively in 384 well plates and was higher in EMTs. Verapamil, Dofetilide, Ivabradine, and Doxorubicin completely blocked voltage and calcium signaling at the highest concentrations tested in 4/4 replicates. BMS-986094, Sunitinib, Lidocaine, Bepridil, and Metoprolol significantly reduced the frequency and/or amplitude at intermediate concentrations. We have designed a novel system capable of high-throughput screening in 2D cultured cells in 384 well plates or leveraging the complexity of 3D cellular models in 24 well plates for increased physiological relevance. The Nautilus platform will provide a stand-alone tool capable of screening significant numbers of compounds for the rapid safety evaluation of drug candidates thereby accelerating drug discovery and development.

Keywords: cardiac safety, CIPA, stem cells, automated imaging, electrophysiology

P1-1

The association between duration of driving and low back pain among online taxibike drivers in Central Jakarta, Indonesia

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Background: Low Back Pain (LBP) is a musculoskeletal disorder in the form of pain in the lower back caused by various factors. One of the common factors is work. Work factors that can cause LBP are body position, posture, workplace design, repetition, duration of work, and work that forces labor. One of the risky jobs is online taxi bike driver, one of Jakarta's most popular transportation types. By using an application to call, an online taxi bike not only acts as a means of transporting people or goods but can also be used to buy goods and order food. The prolonged sitting position in static conditions and limited space to move around contribute to LBP in taxi bike drivers. Therefore, the researcher wants to know the association between driving duration and LBP in taxi bike drivers.
Methods: This was an observational analytic study with a cross-sectional approach on 63 male online taxibike drivers aged 25-35 with normal BMI who met the inclusion and exclusion criteria. The study was conducted in the Central Jakarta area in November 2019. The sampling technique is accidental sampling. Duration of the driving questionnaire and Nordic Low Back Pain Questionnaire were used to gather the data. A bivariate analysis test was conducted with a chi-square statistical test with $p \leq 0,05$.
Results: Thirty-eight online taxibike drivers (60.32%) reported having LBP in the last 12 months. A chi-square statistical test was used, and the results found that the p-value equals 0,414 between driving duration and incidence of LBP.
Conclusion: There is no association between driving duration and LBP in online motorbike taxi drivers. LBP is most common in drivers driving more than 8 hours daily.

Keywords: duration of driving, online taxibike drivers, low back pain, prolonged sitting, static condition

P1-2

Pre-exercise *Cordyceps* supplementation accelerates muscle stem cell replenishment following exercise

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Background: Precursor bone marrow-derived stem cells is required for effectively replenishing satellite cells to accelerate the resolution of muscle inflammation induced by exercise. Stimulating the immune response prior to exercise may contribute to the onset of the tissue repair. This study aimed to reveal pre-exercise immune stimulatory effect by *Cordyceps* on the repairing muscle damage following high intensity aerobic exercise in human skeletal muscle.
Methods: A randomized double-blind placebo-controlled crossover study was performed in the study. A total 14 young sedentary adults (age: 24 ± 0.8 y, BMI: 23 ± 0.6 kg/m^2) were randomly assigned under placebo or Cordyceps supplementation one night (500 mg) and (500 mg) 1 h prior to high intensity intermittent exercise (HIIE, 120% W_{max}) on a cycloergometer. Vastus lateralis muscles were biopsied before, 3 h, and 24 h after exercise challenges.
Results: A significant muscle necrosis (+150%) was observed 3 h after HIIE and this damage response was attenuated during Cordyceps trial (+0.7%). CD34$^+$ cells were more concentrated in the necrotic area. Interestingly, Pax7$^+$ cells contributed to ~10% of CD34$^+$ cells in necrotic muscle area according to immunohistochemical co-staining (Pax7$^+$CD34$^+$), suggesting a replenishment of satellite cells from bone marrow stem cells to damaged site of muscle tissues. Pax7$^+$ cells were homogeneously distributed across non-necrotic area of muscle cross-section. We found a moderate increase in CD34$^+$ bone marrow stem cell infiltration (+36%, $d = 0.5$) in muscle tissues 24 h after HIIE. Pre-exercise CS supplementation accelerated this response to a similar level (+43%, $d = 0.7$) at 3 h. Pax7$^+$ satellite cells in muscle tissues increased 3 h after exercise for PLA- (+57%, $d = 0.8$) and CS-supplemented (+88%, $d = 0.9$) conditions. There was no change on the Pax7$^+$CD34$^+$ ratio across the time point muscle biopsies in both CS and PLA groups. Increasing VEGF mRNA and CD4$^+$ cells with similar extent between CS and PLA ($p < 0.05$) were observed in 3 h following HIIE.
Conclusion: CD34$^+$ cells are required to maintain the state of Pax7$^+$ cells after exercise-induced muscle damage. CD34$^+$ cells partly contributed on to resolve the inflammation in the early phagocytic phase. Other cell (such as stromal cells, fibro/adipogenic progenitor cells, and macrophage) may also contribute on the resolution of inflammation.

Keywords: bone marrow stem cells, satellite cells, immune cells, endothelial progenitor cells

P1-3

CASQ1 as an active partner of stromal interaction molecule 2 in skeletal muscle

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Calsequestrin 1 (CASQ1) in skeletal muscle buffers and senses Ca^{2+} in the sarcoplasmic reticulum (SR). CASQ1 also regulates store-operated Ca^{2+} entry (SOCE) by binding to stromal interaction molecule 1 (STIM1). Abnormal SOCE and/or abnormal expression or mutations in CASQ1, STIM1, or STIM2 are associated with human skeletal, cardiac, or smooth muscle diseases. However, the functional relevance of CASQ1 along with STIM2 has not been studied in any tissue, including skeletal muscle. First, in the present study, it was found by biochemical approaches that CASQ1 is bound to STIM2 via its 92 N-terminal amino acids (C1 region). Next, to examine the functional relevance of the CASQ1-STIM2 interaction in skeletal muscle, the full-length wild-type CASQ1 or the C1 region was expressed in mouse primary skeletal myotubes, and the myotubes were examined using single-myotube Ca^{2+} imaging experiments and transmission electron microscopy observations. The CASQ1-STIM2 interaction via the C1 region decreased SOCE, increased intracellular Ca^{2+} release for skeletal muscle contraction, and changed intracellular Ca^{2+} distributions (high Ca^{2+} in the SR and low Ca^{2+} in the cytosol were observed). Furthermore, the C1 region itself (which lacks Ca^{2+}-buffering ability but has STIM2-binding ability) decreased the expression of Ca^{2+}-related proteins (canonical-type transient receptor potential cation channel type 6 and calmodulin 1) and induced mitochondrial shape abnormalities. Therefore, in skeletal muscle, CASQ1 plays active roles in Ca^{2+} movement and distribution by interacting with STIM2 as well as Ca^{2+} sensing and buffering.

Keywords: CASQ1, STIM2, SOCE, skeletal muscle

Poster

Poster

P1-4

Tripartite motif-containing protein 32 regulates Ca^{2+} movement in skeletal muscle

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Mutations in tripartite motif-containing protein 32 (TRIM32), especially in NHL repeats, have been found in skeletal muscle in patients with type 2H limb-girdle muscular dystrophy (LGMD2H). However, the roles of the NHL repeats of TRIM32 in skeletal muscle functions have not been well addressed. In the present study, to examine the functional role(s) of the TRIM32 NHL repeats in skeletal muscle, TRIM32-binding proteins in skeletal muscle were first searched using a binding assay and MALDI-TOF/TOF. Sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase 1a (SERCA1a) was found to be a TRIM32-binding protein. Next, a deletion mutant of TRIM32 missing the NHL repeats (NHL-Del) was expressed in mouse primary skeletal myotubes during myoblast differentiation into myotubes. Ca^{2+} movement in the myotubes was examined using single-cell Ca^{2+} imaging. Unlike wild-type (WT) TRIM32, NHL-Del did not enhance the amount of Ca^{2+} release from the sarcoplasmic reticulum (SR), Ca^{2+} release for excitation-contraction (EC) coupling or extracellular Ca^{2+} entry via store-operated Ca^{2+} entry (SOCE). In addition, even compared with the vector control, NHL-Del resulted in reduced SOCE due to reduced expression of extracellular Ca^{2+}-entry channels. Therefore, by binding to SERCA1a via its NHL repeats, TRIM32 may participate in the regulation of Ca^{2+} movement for skeletal muscle contraction. Functional defects in TRIM32 due to mutations in NHL repeats may be pathogenic toward LGMD2H.

Keywords: LGMD2H, NHL repeats, SERCA1a, skeletal muscle, TRIM32

P1-5

Protective effects of decoction of *paeonia lactiflora* and *glycyrrhiza uralensis* on muscle atrophy

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Dexamethasone, an artificial glucocorticoid, is used as an anti-inflammatory or immune-suppressive agent. However, chronic use of DEX frequently induces muscle weakness that is related with a slow loss of muscle protein. Our preliminary transcriptome-based pathway analysis of C2C12 myotube treated with an extract of herbal medicine, Jakyak-gamcho-tang (JGT), showed an elevated myogenic gene expression profile including mTOR pathway. Such myogenic or myo-protective potential of JGT was further validated in DEX-treated C2C12 myotubes or experimental mice exposed to repeated high dose of DEX. A significant muscle protein degradation or muscular tissue damages were observed in DEX-treated C2C12 myotubes and in muscle tissues of muscle atrophic mice. Pre- or co-treatment of JGT inhibited the DEX-induced myotube degradation and preserved muscle tissues from DEX toxicity. At the molecular levels, such myo-protective effect of JGT was associated with preservation of mitochondrial oxidative phosphorylation, up-regulation of myogenic signaling pathway like Akt/mTOR/FoxO3 pathway. In addition, JGT inhibited Fbxo32-MuRF1 ubiquitin-mediated muscle protein breakdown and downregulated inflammatory and apoptotic pathways induced by DEX, suggesting that JGT could reverse DEX-mediated muscle damages in multiple pathways. Interestingly, differential transcriptomic analysis of muscle tissues showed that restoration of DEX-induced molecular disturbance by JGT were more pronounced in soleus muscles than in gastrocnemius muscles. Overall, the present study demonstrates that transcriptomic pathway analysis of herbal medicine can be an efficient *in silico* tool to predict novel pharmacological values and elucidate the molecular mechanism of a traditional herbal medicine comprising a diverse biological active ingredients. We also suggest that JGT has a pharmaceutical potential to prevent the muscle atrophy induced by glucocorticoid therapy.

Keywords: herbal medicine, muscle atrophy, transcriptome, pathway analysis, dexamethasone

P1-6

Differential gene expression analysis by RNA-seq data in catalase knockout mice

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There is a dynamic balance between the production and removal of reactive oxygen species (ROS) and reactive nitrogen species (RNS) under normal physiological conditions. However, when the oxidizing product exceeds the antioxidant defense capacity, the body enters an oxidative stress state. Due to the contraction activity, high oxygen consumption rate, and metabolic rate, skeletal muscle significantly produces moderate levels of free radical species such as ROS and RNS. Myogenesis is an important process of maintaining skeletal muscle homeostasis and its physiological function. Forty-one-week-old catalase knockout (CKO) mice with C57BL/6J background exhibited more body weight gain compared to age-matched wild-type (WT) C57BL/6J control mice. In contrast, soleus muscle atrophy was more pronounced in CKO mice compared to WT mice. Gene expression in the soleus muscle in the 41-week-old mice was analyzed using RNA-Seq and validated using qPCR and western blot. RNA-Seq analysis revealed that two genes (gene A, *gene B*) were downregulated and two genes (gene C, *gene D*) were upregulated in the CKO soleus muscle. These findings indicate that *gene A* and *gene B* could be candidate genes critical for the myogenic maintenance of skeletal muscle against the free radical-induced atrophy.

Keywords: skeletal muscle, sarcopenia, free radical, catalase, RNA-seq

P1-7

Hibernation-induced muscle atrophy resistance in the skeletal muscles of the Syrian hamster

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Background: Hibernating animals have an unexplored physiological function of skeletal muscle atrophy resistance, in which muscle loss is minimal despite experiencing prolonged periods of inactivity and malnutrition. In this study, we examined the molecular mechanisms of skeletal muscle atrophy resistance induced by hibernation with using a mammalian hibernator, the Syrian hamster.
Methods: The Syrian hamsters (male, 11 weeks old) were exposed to a short-day cold environment (8h Light: 16h Dark, ambient temperature 5°C) to induce hibernation. Sciatic nerve transection was used as an experimental model of skeletal muscle atrophy, and the contralateral side was the control group by sham-operation. The skeletal muscle samples were collected following 14 days of surgical intervention and were used for biochemical and histological analysis. All experimental procedures and animal care performed in this study were approved by the Animal Care and Use Committee of the Hiroshima University (A22-7-3) and the Fukuyama University (2022-A-12).
Results: Whereas the non-hibernating hamsters showed a significant reduction in skeletal muscle mass following 14 days of denervation, skeletal muscle weight and muscle fiber cross-sectional area were almost completely unaffected with denervation in the hibernating hamsters, indicating that skeletal muscle atrophy was prevented. Comprehensive gene expression analysis using RNA-sequencing identified a total of 392 hibernation-inducible differentially expressed genes (DEGs) in skeletal muscle. Furthermore, among these DEGs, we have narrowed down the set of genes that show a unique alteration of gene expression with hibernation (activated/suppressed by hibernation) and have obtained several candidate genes that ensure skeletal muscle atrophy resistance.
Conclusion: These results suggest that skeletal muscle atrophy resistance in hibernating mammals is a plastic remodeling mechanism that is induced during hibernation, rather than an innate biological function that certain hibernating species, including hamsters, constantly exhibit.

Keywords: Syrian Hamsters, skeletal muscle atrophy, denervation

P1-8

Isolation of skeletal muscle stem cells from a mammalian hibernator Syrian hamster

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Background: Satellite cells (SCs) are the primary stem cells in adult skeletal muscle and play an important role in skeletal muscle growth and regeneration. SCs are widely recognized for their contribution to the maintenance of skeletal muscle mass, and declines in skeletal muscle and SCs content due to aging and long-term inactivity are associated with increased risk of diabetes, sarcopenia, and higher mortality rates. In contrast, hibernating animals are able to maintain skeletal muscle mass despite experiencing prolonged period of inactivity. Skeletal muscle stem cells, SCs potentially contribute to this muscle mass maintenance machinery, however, no studies have isolated SCs from the skeletal muscle of hibernating animals. In this study, we aimed to establish a method to isolate SCs from skeletal muscles of the small hibernating mammals, the Syrian hamsters.
Methods: The male Syrian hamsters (Slc:Syrian) aged 4-6 months were used in this study. Skeletal muscle samples were collected from hamster hindlimbs and SCs were crudely isolated by collagenase treatment. The Re-plating method was then used to purify and proliferate SCs to reduce fibroblasts contamination. Purified SCs by the Re-plating method were stained with immunofluorescent techniques, and the percentage of myogenic cells (Pax7-positive/MyoD-positive) was calculated. The isolated SCs were then treated with differentiation medium to confirm the differentiation potential of the myogenic cells. All experimental procedures and animal care performed in this study were approved by the Animal Care and Use Committee of the Hiroshima University (A22-7-3) and the Fukuyama University (2022-A-12).
Results: The percentage of myogenic cells in the crude purified cells was 34.1±3.2%, with many fibroblasts contaminated. In contrast, high purity was confirmed with over 95% of the cells isolated after purification by the Re-plating method were myogenic cells. Further, differentiation induction of the isolated SCs resulted in cell fusion and formation of myotubes, confirming that they maintained their potential as myogenic cells.
Conclusion: We have established a method for isolating SCs from skeletal muscle of the hibernating animals. The Re-plating method is effective in isolating SCs from skeletal muscle of hibernating animals with high purity and relative ease. In the future, we will apply this method to investigate the involvement of SCs in the mechanism of acquiring resistance to muscle atrophy in hibernator and the changes in proliferative and differentiation ability of SCs associated with hibernation.

Keywords: Syrian Hamsters, satellite cells, proliferation, differentiation

P1-9

Dorsomorphin, an AMP kinse inhibitor, inhibits skinned smooth muscle contraction through supression of myosin light chain phosphorylation

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AMP kinase is known to inhibit smooth muscle contraction through inhibition of myosin light chain kinase (MLC kinase) activity (e.g. Horman et al, J. Biol Chem 2008, 283;18505–12). On the other hand, it is still unclear whether dorsomorphin, an inhibitor of AMP kinase, has any effects on smooth muscle contractility. To clarify effects of dorsomorphin on contractile elements in smooth muscle, we investigated the agent effects on skinned (cell membrane permeabilized) smooth muscle contraction. Cell membrane and intracellular Ca^{2+} store of small strips of taenia cecum from guinea pig were permeabilized with beta escin, and Ca ionophore A23187, respectively. Surprisingly, dorsomorphin at 10 micro M and higher significantly reduced Ca^{2+} induced force development of skinned taenia cecum to decrease Ca^{2+} sensitivity for the force. Also, tautomycin, a potent and selective inhibitor of myosin phosphatase attenuated the inhibitory effects of dorsomorphin on Ca^{2+} induced force development of skinned taenia cecum. Furthermore, in thiophosphorylated skinned preparations, dorsomorphin did not affect ITP induced

force development. These results suggest that dorsomorphin seems to suppress contractile element activity though inhibition of activity of myosin phosphatase.
COI:No.

Keywords: smooth muscle, myosin light chain, phosphorylation, AMP kinase, skinned preparation

P1-10

How do functional responses to antimuscarinic medications in juvenile or adult bladder detrusor tissues differ?

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Background: The first-line pharmaceutical therapies for managing overactive bladder (OAB) are antimuscarinics. Their main mechanism of inhibiting spontaneous contractions in the urinary bladder during the filling phase by blocking the action of acetylcholine in the detrusor, has been identified in juvenile models. The prevalence of OAB increases with age and it is not clear whether this is due to changes in the urinary bladder tissue structure itself, alterations in lifestyle or other causes. In order to assess the changes to urinary bladder itself, this study aims to find the differences in the ability of commonly prescribed antimuscarinics to inhibit contractions of the detrusor and compare these responses in juvenile and adult porcine tissues.
Methods: Strips of porcine U&LP or detrusor from the adult or juvenile model were mounted in carbogen-gassed Krebs-bicarbonate solution at 37°C. The tissues were paired with carbachol concentration-response curves performed in the absence or presence of oxybutynin (1μM), solifenacin (1μM) darifenacin (100nM), tolterodine (1μM), trospium (100nM) and fesoterodine (100nM). Concentrations were chosen to ensure complete concentration-response curves in response to carbachol. pEC50 values for each curve were analysed and estimated affinities calculated. Ethical approval was not required for this study as tissues were sourced from the local abattoir after slaughter for the routine commercial provision of food.
Results: A right parallel shift was produced from the control in the juvenile detrusor by (n value; estimated affinity) included oxybutynin (10, 7.47) solifenacin (8, 7.87), darifenacin (11, 7.58), tolterodine (8, 8.09), trospium (8, 8.69) and fesoterodine (8, 8.67). A right parallel shift was produced from the control in the adult detrusor by (n value; estimated affinity) included oxybutynin (8, 7.21) solifenacin (8, 6.63), darifenacin (9, 7.95), tolterodine (8, 7.78), trospium (9, 9.30) and fesoterodine (8, 8.55). A right parallel shift was produced from the control in the adult detrusor by (n value; estimated affinity) included oxybutynin (10, 7.47) solifenacin (8, 7.87), darifenacin (11, 7.58), tolterodine (8, 8.09), trospium (8, 8.69) and fesoterodine (8, 8.67). Comparisions of estimated affinities of each antimuscarinic between juvenile and adult tissues revealed no differences in each tissue's functional response to the six antimuscarinics ($p > 0.05$).
Conclusion: Although preliminary, with this study ongoing, there appears to be no significant differences between detrusor functional responses to antimuscarinics of differently aged porcine samples. Further supporting that these medications can assist in the treatment of OAB and lower urinary tract symptoms in the detrusor layer. Differences in compliance may be due to lifestyle or behavioral changes with age rather than alterations in the tissue's ability to respond to the prescribed medication themselves.

Keywords: bladder, antimuscarinics, overactive bladder, detrusor, smooth muscle

P1-11

Protective effects of ginsenoside Rc on hydrogen peroxide-induced muscle damage

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Background: Loss of skeletal muscle mass and function reduces the quality of life and increases morbidity and mortality, thereby emerging as a serious social problem in the aging society. Recently, many studies have been reported to promote muscle differentiation and muscle function by enhancing mitochondrial function. Ginsenoside Rc is a major component of ginseng and has been reported to have various pharmacological activities, but little research has been done on muscle loss.
Methods: In this study, we examined the effects of gRc on H_2O_2-induced reduction of cell viability both in C2C12 myoblasts and myotubes, and H_2O_2-induced myotube degradation. In addition, we also investigated the effects of gRc on the production of intracellular reactive oxygen species and mitochondrial superoxide, ATP generation, and peroxisome proliferator-activated receptor-gamma co-activator 1É' activity both in myoblasts and myotubes under H_2O_2 treatment condition. Furthermore, to verify the mechanism of action of gRc, we performed transcriptome analysis for myotubes treated with or without gRc under H_2O_2 treatment condition.
Results: We found that gRc effectively suppressed HO-induced cytotoxicity, production of intracellular ROS and mitochondrial superoxide, restored PGC-1É' promoter activity, and increased ATP synthesis. We also found that in C2C12 myotubes, gRc maintains mitochondrial mass and biogenesis and inhibits muscle degradation, thereby having potent muscle-preserving effects under conditions of oxidative stress, as supported by transcriptomic analysis.
Conclusion: In this study, we confirmed the possibility that gRc can be developed as an effective treatment for muscle loss and muscle weakness. Therefore, we plan to conduct follow-up studies on the preventive and therapeutic effects of gRc in various pathological conditions of muscle loss.

Keywords: Panax ginseng, oxidative stress, mitochondrial biogenesis, muscle atrophy, transcriptome

P1-12

Hibernation delays the regenerative processes in the skeletal muscle of the mammalian hibernator Syrian hamster

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Background: While skeletal muscle are markedly atrophied following

prolonged periods of inactivity, hibernating animals exhibit minimal skeletal muscle atrophy despite experiencing approximately six months of inactivity during hibernation. The higher regenerative capacity of skeletal muscle is critical for the maintenance of muscle mass, however to our knowledge, no previous studies have evaluated the regenerative capacity of skeletal muscle in hibernating animals. In this study, we examined the effects of hibernation on the regenerative processes of skeletal muscle in the hibernating mammals, the Syrian hamster.
Methods: The Syrian Hamster/SLC, as an animal model of hibernation, was induced to hibernate by exposure to a cold short-day environment (ambient temperature 5°C, 8 hours light/16 hours dark), while the control group was kept in a normothermic long-day environment (ambient temperature 22°C, 12 hours light/12 hours dark). After confirming the continuation of hibernation cycles (6~8 cycles) consisting of deep hibernation lasting 3~4 days and periodic arousal for about 1 day, cardiotoxin (CTX) was administered to the tibialis anterior muscle (10 μM, 200 μl) during periodic arousal phase and pharmacological muscle damage was induced. The contralateral tibialis anterior muscle was treated with an equal volume of phosphate-buffered saline. The individuals that continued the hibernation cycle following the intervention were included in the analysis, and the tibialis anterior muscle was sampled 14 days after CTX administration for histochemical analysis. All experimental procedures and animal care performed in this study were approved by the Animal Care and Use Committee of the Hiroshima University (A22-7-3) and the Fukuyama University (2022-A-12).
Results: In the non-hibernating group, regenerating myofibers with a centrally located nuclei were identified 7 and 14 days following CTX administration. In contrast, regenerating myofibers with central nuclei were almost absent in the skeletal muscle of the hibernating animals on 14 days of CTX administration, and mononuclear cells, primarily inflammatory cells, infiltrated the interstitial region of muscle fiber, indicating an apparent failure of muscle regeneration.
Conclusion: It was demonstrated that hibernation severely delays the regenerative process of skeletal muscle following CTX-induced muscle damage. This may be due to the suppression of acute inflammation such as delayed infiltration of inflammatory cells such as macrophages and delayed skeletal muscle regeneration due to suppression of myogenic program of satellite cells.

Keywords: Syrian Hamster, hibernation, skeletal muscle, macrophage, satellite Cells

P1-13

Musculoskeletal pain and occupational ergonomic profiles among laboratory technicians in indonesia: a preeliminary study

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Prolonged working time and high accuracy demand among laboratory technician frequently resulted musculoskeletal pain. Ergonomic position plays an important to anticipate musculoskeletal pain among laboratory technicians. This study aimed to determine the prevalence of musculoskeletal pain as well as daily ergonomic profiles among laboratory technician in Indonesia setting. A total of twenty laboratory technician inquired to fill a Nordic Musculoskeletal questionnaire. Sixty percents of participants were male. The range of all participants were 34-54 years old. About 70% of participants reported musculoskeletal pain. From those number the absent of seat back during sitting produced pain in 60% male and 67% of female participants. The participant with duration of 2-4 hours and 5-6 hours in sitting position showed 87% and 100% pain experience respectively. The duration of standing position in 2-4 hours and 5-6 hours/day give 94% and 75% pain among participants. The most dominant pain location was reported in the neck, shoulders, and knees. This study suggested the important of ergonomic position adjustments during work among laboratory technician to attenuate the musculoskeletal pain.

Keywords: muscle, analyst, pain, health, quality of life

P1-14

Muscle-specific loss of prolyl hydroxlase domain family (PHDs) induces reduction of exercise capacity and weight loss

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Oxygen consumption is highly increased in muscle during its excitation and contraction. The prolyl hydroxylase domain family (PHD1, -2, -3, encoded by *Egln*2, 1, and 3) are dioxygenases that hydroxylate proline residues of their specific substrates. It is well known that under normoxia, PHDs hydroxylate hypoxia-inducible factors (HIF) and decrease the HIFs stability. While signaling initiated by hypoxia largely regulate cellular metabolism, the physiological role of PHDs in myocyte is poorly understood. Here, we used muscle-specific PHD1/2/3-null (PHD MTKO) mice to investigate the roles of these PHDs as well as HIF in exercise-related or dietary pathway. Loss of PHDs elevates stability of HIF1α protein and significantly increases its target genes in the muscle. Although PHD MTKO mice showed high activation of myogenesis and erythropoiesis, these mice also showed dramatical decrease in exercise capacity. This low exercise performance was further supported by mitochondrial contents that were highly repressed in muscle of PHD MTKO mice. Surprisingly, PHD MTKO mice showed drastic decrease in body weight when they received high-fat-diet. Thus, these findings demonstrate the pivotal role of muscular PHDs in exercise capacity and weight loss during obesity.

Keywords: muscle, PHD, HIF1a, hypoxia

P2-1

Influence ingestion of red dragon fruit extract on mitochondrial cytochromes soleus muscle after moderate exercise in rats (Rattus norvegicus)

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Background: Ingestion red dragon fruit (RDF) antioxidants can suppress free radicals characterized by decreased levels of cytochrome enzymes, and the release of cytochrome-c from mitochondria to the cytosol does not occur. Hence, the process of apoptosis runs slowly. Mild exercise causes an increase in free radicals, which causes oxidative stress, which causes damage to cell function to mitochondrial dysfunction and plays a role in the regulatory process of cytochromes as an

induction of apoptosis which normally cannot be released from mitochondria. This study aims to determine the antioxidant potential of RDF in delaying oxidative stress, which increases the function of mitochondrial cells in the soleus muscle.
Methods: This study involved 25 three-month-old male mice with an average weight of 200 g. Mice were randomly allocated into five groups as follows: two control groups (K1 [no exercise, no RDF] and K2 [exercise, no RDF] and three groups (P1, P2, and P3; subjected to exercise and treated with 75, 150, and 300 mg kg-1 body weight RDF, respectively). The exercise was performed by swimming for 20 minutes three times a week for 31 days. Cytochrome-C and MDA were measured via ELISA, and a histopathological examination was muscle hematoxylin and eosin.
Results: Comparison of MDA levels after administration of RDF extract between K2 and P1, P2, and P3 The results showed a significant difference ($p<0.05$ for P1 and P2, and $p<0.01$ for P3), cytochrome-C levels were compared between K2 (16.5 ng/m g protein) and P1 (13.6 ng/mg protein), P2 (11.3 ng/mg protein), and group P3 (6.7 ng/mg protein). The results showed a significant difference ($p<0.05$ for T1 and T2, and $p<0.01$ for T3).
Conclusion: Red dragon fruit (antioxidant) in sports suppresses oxidative stress on cytochrome-c so that the apoptotic signal occurs significantly in the muscles, can prevent the apoptotic process, reduce tissue damage, cause repair of cell function, and mitochondria can work typically. If there are many cytochromes in the cytosol, this indicates cell dysfunction.

Keywords: Red dragon fruit (RDF) extract, strenuous exercise, cytochrome-c, soleus muscle

Poster

P2-2

Effect of different intensities aerobic exercise to cardiac angiogenesis regulation on wistar rats

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The Adaptation response of myocardium angiogenesis stimulated by specific exercise intensities remains unclear. The aims if this study to explore the effect of different intensities aerobic exercise to cardiac angiogenesis via HIF-1α, PGC-1α, VEGF and $CD34^+$ in Wistar rats.
Wistar rats are devided into control and exercise groups. Exercise groups were trained on a treadmil for 12 weeks, 30 min/ day, for 5 days with low, moderate and high-intensity groups. The rats were sacrified and the myocardium was collected and preserved at - 80°C until used. Cardiac protein samples were extracted and run for Western blotting using specific antibodies : hypoxia-inducible factos (HIF)-1α, PGC-1α, VEGF and $CD34^+$.
Results showed that protein expression of HIF-1α, PGC-1α, VEGF and $CD34^+$ was increased significantly by different intensities the exercise group compared to the control. A correlation statistics test showed that there was strong correlation effect of HIF-1α and VEGF protein expression in low ($p=0.047$) and high-intensities group ($p=0.009$), but no significant effect of HIF-1α on VEGF in the moderate groups. However evidently, in moderate group, VEGF protein was strongly effect by PGC-1α ($p=0.037$), but no correlation effect of PGC-1α on VEGF in other groups.
In conclusion, different exercise intensities induce a different modulation pattern of proteins which might be responsible for cardiac adaptation, especially angiogenesis.

Keywords: angiogenesis, exercise, HIF-1α, PGC-1α, myocardium

P2-3

The anti-aging effects of exercise training on gut microbiota diversity and composition in mice

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The gut microbiota is implicated in various diseases, highlighting the importance of maintaining a healthy microbial balance. Recent studies suggest that aging can negatively influence the stability and diversity of the gut microbiome, which would be associated with increased frailty, cognitive decline, and reduced physical function. Whereas, exercise training is proposed to have a positive impact on the gut microbiome, promoting diversity and the presence of beneficial bacteria. Nevertheless, if exercise training can prevent or reverse the aging-associated changes in the gut microbiome is unknown. Thus, this study aimed to investigate the impact of aging and exercise training on gut microbiota diversity and composition in mice. Fecal samples were collected from young (8-wk old) and old (18-mo old) male C57BL/6N male mice (n=5/age) before and after exercise training which was composed of continuous running at 65% of maximal speed 5 days/wk at a 10° incline on a rodent treadmill. As a result, exercise capacity was improved in both groups, accompanied by substantial weight loss only in the old mice. Aging was characterized by reduced α-diversity, increased abundance of Lactobacillaceae and Bacilli, and decreased levels of Clostridia. However, the exercise training in old mice led to increased α-diversity, decreased Bacilli, and increased Clostridia, suggesting the anti-aging effects on the gut microbiome. Although exercise training did not induce substantial changes in the microbiome diversity in young mice, a difference in β-diversity was present in young mice before and after exercise training, which is also shown in old mice with less extent. These findings contribute to our understanding of the intricate relationship between the microbiome, aging, and exercise training, shedding light on the potential role of exercise training to modulate the gut microbiome and promote healthy aging.

Keywords: gut microbiome, aging, exercise, mouse

P2-4

The epigenetic regulation of exercise training in vascular fibrosis with aging

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While aging is known to cause structural remodeling in the vasculature, exercise has been suggested to prevent these aging effects. However, these potential anti-aging effects of exercise at the molecular level are not well understood. In this study, we tried to clarify the epigenetic mechanisms of detrimental aging effects and the anti-aging effect of exercise on the vasculature, focusing on histone modification. As a result, it was found that methylation at lysine 27 in histone variant H3 (H3K27me) decreased and acetylation at lysine 27 in histone variant H3(H3K27ac) complementarily increased as aged in mouse aortas. Also, we found transcriptional changes of several genes associated with vascular fibrosis with aging. In addition, the enrichment of H3K27ac increased in the transcriptional regulatory region (promoter) of a fibrosis gene, therefore proposing the epigenetic mechanism for vascular fibrosis with aging through histone modification. On the contrary,

aging-associated changes in H3K27ac and H3K27me were mitigated by an acute exercise or long-term exercise, and increased enrichment of H3K27ac on the transcriptional regulatory region of a fibrotic gene was ameliorated. This result can explain, partly, the decreased expression level of aging genes with exercise. Together, our findings suggest the epigenetic mechanism of the anti-aging effect of exercise on the vasculature, therefore warranting a future study using large animals or clinical studies. Ultimately, these findings could contribute to the development of new anti-aging therapies.

Keywords: histone modification, vasculature, aging, exercise training, fibrosis

P2-5

Experience of endurance exercise training augments the hypertrophic effects of exercise in plantaris muscle of re-trained rats

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Background: In general, skeletal muscle adaptations in response to exercise training are lost when the training stops (de-training). Several studies reported that resistance exercise training experience enhances hypertrophic responses in skeletal muscle following re-training. This phenomenon is called as "muscle memory" and/or "epigenetic memory". However, memory effects of endurance exercise training on skeletal muscle adaptations following re-training remains unknown. The aim of the present study was to investigate the effects of endurance exercise training experience on skeletal muscle adaptations in response to subsequent re-training.
Methods: Male Wister rats, aged 8 weeks, were randomly divided into three groups: 6 weeks of endurance training (ET, week 8-14), 6 weeks of de-training (EDT, week 14-20), or 6 weeks of re-training (ERT, week 20-26). The training groups were subjected to treadmill running exercise (40 m/min, 60 min/day, 5 days/week) for 6 weeks. Approximately 48 h after the last training session, plantaris muscles were dissected. Age-matched sedentary rats were used as a control (Con). Immunohistochemical analysis was carried out to identify muscle fibers and myonuclei, and individual muscle fiber cross-sectional area (mCSA) and myonuclear number were measured. Whole protein fraction was also isolated for Western blot analysis.
Results: There were no significant effects of the first endurance training on plantaris muscle mass, mCSA, and myonuclear content. However, relative plantaris weight, mCSA, and myonuclear content were significantly higher in ERT than in age-matched Con groups ($P < 0.05$). The hypertrophy rate of plantaris muscle was also significantly greater in ERT than in ET groups ($P < 0.05$). Protein levels of heat shock protein (HSP) 72 was increased significantly in the ET group ($P < 0.05$), and then returned to the age-match Con value after the EDT period. Surprisingly, the protein expression of HSP72 in the ERT group was significantly higher than that in both age-matched Con and ET groups ($P < 0.05$). Similar trend was also observed in the expression of HSP25.
Conclusion: These results suggested that endurance exercise training experience promotes hypertrophic effects in response to subsequent re-training. A part of HSPs may play a key role in this phenomenon.

Keywords: endurance exercise, muscle hypertrophy, epigenetics, muscle memory, heat shock protein

P2-6

Role of stingless bee honey on blood glucose and inflammation marker in rat's skeletal muscle

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Stingless bee honey is known as an anti-inflammatory agent. Exercise may affect some inflammatory marker increases. This study investigates the effect of stingless bee honey on blood glucose levels and inflammatory markers IL-6 and TNF-α in rat's skeletal muscle. 20 Wistar rats are divided into 4 groups (control group, honey group, exercise control group, and exercise with honey group). Stingless bee honey was given 9.3gr/BW every morning, one hour before exercise). The exercise group was set to swimming exercise in a 1mx1mx80cm pool with a temperature 35 degrees for 6 days/week full 28 days. Gastrocnemius muscle was collected to analyze the inflammatory marker IL-6 and TNF-α with western blot. The significant finding was followed, first, there was no significant decrease in blood glucose of Wistar rats. Second, there was a significant increase in IL-6 and TNF-α in the exercise group control. Third, there was a significant decrease in IL-6 and TNF-α in exercise with a honey group. In conclusion, stingless bee honey did not affect the blood glucose but affected the inflammation process because of exercise.

Keywords: exercise, inflammatory marker, stingless bee honey, wistar rats

P2-7

Dynamics of mRNA expression in rat skeletal muscle after resistance exercise

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Background: mRNA expression changes in skeletal muscle after resistance exercise but not only increases or decreases in mRNA levels but also dynamics (temporal patterns) of those are important for different cellular outputs. However, little is known about the dynamics of mRNA expression after resistance exercise. The purpose of this study was to clarify the dynamics of mRNA expression in rat skeletal muscle after resistance exercise.
Methods: We used 11 weeks of male Wistar rats (total 18). As resistance exercise, isometric contraction was performed by electrical stimulation to the right tibialis anterior (TA) muscle under the isoflurane anesthesia. Rats performed 10 times 3 seconds of 100Hz muscle contraction with 7 seconds intervals. This protocol was repeated 5 sets with 3 minutes intervals. Rats were divided into 6 groups that collected TA muscles before and 0, 1, 3, 6, and 12 h after the exercise. We isolated total RNA from the right TA for RNA sequencing ($n = 3$ each group). Transcript per kilobase million (TPM) was calculated as RNA content values. To identify differential expressed gene (DEG), we performed one-way ANOVA and calculated q values by the Benjamini-Hochberg method ($q < 0.05$ as the significance threshold). Furthermore, DEGs were identified as genes that have TPM more than 2.0-fold (up-regulated DEG (UP_DEG)) and less than 0.5-fold (down-regulated DEG (DOWN_DEG)) compared with those at pre-exercise at any time point after the exercise. Gene ontology (GO) analysis was performed using Metascape.
Results: After the resistance exercise, we identified 263 UP_DEGs, 82

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DOWN_DEGs and 4 UP and DOWN_DEGs. We analyzed how quickly genes changed after a resistance exercise using Peak50, which is the time at 50% of the peak. We separated DEGs into fast (Peak50 between pre and 1h), medium (Peak50 between 1h and 3h) and slow (Peak50 between 3h and 12h) groups. The following GO terms were enriched in the UP_DEGs; vasculature development and cellular responses for the fast group, muscle structure development for the medium group, and muscle cell differentiation for the slow group. However, no significant GO terms existed for any group in the DOWN_DEGs. Next, we analyzed the decay of DEG after the peak using back_Peak50, which is the duration from the time at the peak to the time at 50% of the peak after the peak. There were 44 genes with back_Peak50/Peak50 > 2.0, indicating sustained increases after the exercise. As GO terms, response to mechanical stimulus, regulation of transcription from RNA polymerase II promoter in response to stress, blood vessel development and skeletal muscle cell differentiation were enriched for the 44 sustained UP_DEGs. However, significant GO terms were not enriched in 47 transient increased genes, which have back_Peak50/Peak50 < 1.0.
Conclusion: Our transcriptomic analysis revealed that dynamics in genes that increase after resistance exercise differ depending on the biological function of genes and sustained increases in mRNA levels may be an important regulation for exercise adaptation.

Keywords: transcriptome, muscle contraction, temporal pattern, skeletal muscle, acute exercise

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P2-8

Different intensities of exercise modulates liver histopathology appearance and mitophagy gene expression in low protein diet-induced rats

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Background: Malnutrition is still one of the major health issues in Indonesia. Low intake of nutritious food, especially protein, still becomes the common cause of malnutrition. Stress oxidative and mitochondrial disruption have major roles in the alteration of liver metabolism after induction of a low protein diet. One of the ways to prevent them is by changing our lifestyle, particularly by exercise. Different intensities of exercise might modulate different adaptations in the liver. Mitophagy served as one of the molecular pathways to improve mitochondrial damage, in correlation to liver histopathology that is associated with metabolic zonation in the liver. Unfortunately, the optimal intensity for this improvement yet still in question. This study aims to investigate the effect of exercise with different intensities on liver histopathology and mitophagy gene expression in low protein-induced Wistar rats.
Methods: Eight groups of 8 weeks Wistar rats, consisted of control, low protein diet, and 3 groups of different intensities of treadmill exercise (low, moderate, high), as well as 3 groups of different intensities of treadmill exercise after 26 days of low-protein diet/LPD induction (low-LPD, mod-LPD, high-LPD). The exercise was conducted 150 minutes per week, 30 minutes/day, for a whole 8-week period. Liver histopathology scoring with hematoxylin-eosin staining (congestion and cloudy swelling based on metabolic zonation, inflammation, and steatosis), Pink1 and Parkin gene expression by real-time PCR were analyzed.
Results: We found significant differences in congestion (p=0.003), cloudy swelling (p=0.012), and inflammation (p=0.006), but no difference in steatosis (p=0.914). The scoring of congestion, cloudy swelling, and inflammation was significantly increased in low protein diet group compared to the control. Low and moderate intensity exercise significantly reduced congestion and cloudy swelling in low protein diet group. While for cloudy swelling, all intensities were found to significantly decrease it, but only moderate intensity reduced inflammation. We also found a significant difference in Pink 1 (p=0.015), and the tendency of increment in Parkin gene expression. Moderate intensity of exercise significantly increased Pink1 gene expression (p=0.021) after low protein induction.
Conclusion: Among three different intensities of exercise, the moderate intensity has the greatest potential in modulating liver histopathology appearance and mitophagy gene expression in low protein diet-induced Wistar rats.

Keywords: mitophagy, exercise, liver, metabolic zonation, low protein diet

P2-9

Alteration of histopathology, autophagy, and collagen gene expression after different intensities of exercise in wistar rats induced by low protein diet

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Background: A low-protein diet induces malnutrition, characterized by changes in molecular mechanisms involving many organs, including the skin. Stress oxidative plays a major part in malnutrition, and one of the ways to reduce its level is by exercising regularly. Exercise is known to increase blood flow to the skin by discarding ROS and stimulating fibroblasts that eventually produce collagen. Autophagy is a homeostatic mechanism that works in molecular pathways that alters ROS production, cell renewal, and collagen production rate. This study aims to investigate histopathology, autophagy, and collagen gene expression after different intensities of exercise in Wistar rats induced by low-protein diet.
Methods: Thirty-two Wistar rats, aged 8 weeks, were divided into 8 groups: control, low intensity, moderate intensity, and high intensity exercise; low protein diet, low intensity with low protein diet, moderate intensity with low protein diet, and high intensity with low protein diet. Induction of low protein diet was conducted on the first 26 days. The duration of exercise was 30 minutes per day, 5 days per week, for 8 weeks. Skin histopathology was analyzed after hematoxylin-eosin staining. Autophagy and collagen gene expression were measured after quantitative real-time PCR.

Results: Epidermal thickness significantly differed between groups (p=0.012), especially in low intensity with low protein diet (p=0.026) and high intensity with low protein diet (p=0.009) compared to low protein diet group. LC3 gene expression significantly differed between groups (p=0.002), especially between moderate intensity with low protein diet and moderate intensity group (p=0.042), and between high intensity with low protein diet and high intensity group (p=0.021). As for p62 gene expression, we found a tendency of p62 gene expression to increase in all low protein diet groups, while moderate and high intensities of exercise returned the p62 levels toward the baseline. We also examined the PGC1α and Col3a1 gene expression and found a tendency to increase in low and high intensities with low protein diet compared to low protein diet group.
Conclusion: Different intensities of exercise might induce alteration of histopathology, autophagy, and collagen gene expression as a complex mechanism that might be associated with the change in metabolic reactions and stress oxidative, intending to achieve homeostasis in the skin.

Keywords: autophagy, exercise, skin, collagen, low protein diet

P2-10

Mathematical modeling analysis revealed that Akt-mediated inhibition of AMPK is important regulation for resistance exercise-induced protein synthesis in skeletal muscle

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Resistance exercise (RE) activates muscle protein synthesis via the Akt pathway, a major mechanism of protein synthesis, which has two regulatory properties. One is the crosstalk with the AMP-activated kinase (AMPK) pathway, and AMPK and Akt inhibit each pathway. The other is the low-pass filter property revealed by the previous study in which weak, sustained upstream signal of Akt, rather than strong, transient signal, strongly induced downstream signal of Akt using growth factor stimulation to cells. The analysis showed that this could be explained by low-pass filter property of Akt pathway. However, these properties are not well understood in skeletal muscle after RE. Developing mathematical models and performing simulations are powerful tools to clarify the regulatory properties of signaling pathways. Therefore, the purpose of this study was to develop a mathematical model of the Akt pathway in skeletal muscle after RE and to study these pathway properties by simulations. The right tibialis anterior muscle of 11-wk old male Wistar rats performed isometric contraction by percutaneous electrical stimulation. The contraction protocol was 10 times 3 seconds of 1, 10, 20, 60 or 100 Hz muscle contraction with 7 seconds intervals. This protocol was repeated 5 sets with 3 minutes intervals. Muscle samples were collected before and 0, 1, 3, 6 and 12 h after the 100Hz muscle contraction, and 0 and 3h after 1, 10, 20 and 60 Hz muscle contraction. The phosphorylation status of signaling molecules in the Akt and AMPK pathways was measured by Western blotting. The mathematical model was developed using ordinary differential equations by estimating the reaction rate parameters. To investigate the crosstalk between the Akt and AMPK pathways, we investigated the contribution of each crosstalk by simulation with quantitatively changing the inhibition of the AMPK pathway by Akt and the inhibition of the Akt pathway by AMPK. The analysis revealed that p-Akt-mediated inhibition of AMPK is essential for RE-induced protein synthesis. Next, we tested whether the Akt pathway has a low-pass filter property by simulating the phosphorylation of Akt downstream molecules in response to different input patterns with the same input integrals. The peaks of the phosphorylated p70S6 kinase were about 10% higher with transient, high-intensity input than with sustained low-intensity input. These results suggest that the low-pass filter property of the Akt pathway is unlikely to be present in our model and in the range of intensities we examined in this study. Our model provides a tool to systematically study protein synthesis signaling after RE.

Keywords: skeletal muscle, resistance exercise, cell signaling

P2-11

Expression of c-Fos in nausea-associated brain regions during high-intensity endurance exercise

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Background: Exercise-induced nausea is thought to be one of the factors limiting athletic performance, but its neuronal mechanisms remain unclear. This study aimed at determining whether the lateral parabrachial nucleus (LPBN), nucleus tractus solitarius (NTS), and area postrema (AP), which are associated with generalized nausea, get activated during high-intensity endurance exercise (HIE), and further, to characterize the functional roles of the LPBN-NTS and LPBN-AP pathways.
Methods: Firstly, male Wistar rats were subjected to treadmill exercise [low/high-intensity endurance exercise, LIE (~20 m/min) and HIE (~34 m/min)] or Control (CON; 0 m/min) for 90 min to examine neuronal activation, by investigating c-Fos immunohistochemistry in the brain and functional connectivity of the LPBN-AP-NTS network at each exercise intensity. Secondly, to examine whether LPBN-projecting NTS and/or AP neurons are activated during HIE, retrograde tracer (CTb) was injected into the LPBN and the number of cells co-expressing with c-Fos during HIE was counted.
Results: The number of c-Fos positive cells of LPBN, NTS, and AP in the HIE group was significantly higher than CON and LIE ($p<0.05$), and a significant correlation between the LPBN and AP c-Fos positive cells ($r=0.78$, $p<0.05$) was determined. The number of LPBN-projecting AP neurons activated by HIE was significantly higher than the number of LPBN-projecting NTS neurons ($p<0.05$).
Conclusion: These results suggest that the activation of LPBN-projecting AP neurons may enhanced functional connectivity between LPBN and AP regions during HIE. Future studies are required to examine whether this AP-LPBN pathway is involved in the development of exercise-induced nausea.

Keywords: nucleus tractus solitarius, area postrema, lateral parabrachial nucleus, nausea, exercise

P2-12

Effect of previous endurance training experience on skeletal muscle adaptation to endurance training

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Background: It has been suggested that previously trained muscles can acquire strength and volume faster than untrained muscles. This phenomenon known as muscle memory (Snijders et al., 2020). However,

whether muscles that have experienced endurance training acquire endurance capacity faster than untrained muscles have not been well studied (Lindholm et al., 2016; Lee et al., 2018). Therefore, in this study, we examined whether mitochondrial adaptation in skeletal muscle to endurance training differs between untrained mice and those that have experienced endurance training once.
Methods: 4-week-old male Institute of Cancer Research (ICR) mice were used. We assigned all animals into six groups (n = 10~11 each group):4 weeks control group (rest for 4 weeks), 4 weeks training group (training for 4 weeks), control group (rest for 8 weeks), training group (endurance training for 2 weeks following rest for 8 weeks), detraining group (rest for 4 weeks following endurance training for 4 weeks), and retraining group (endurance training for 2 weeks following detraining period). Approximately 24-27 h after their last training, all mice were sacrificed and tibialis anterior, soleus and plantaris muscles were collected. We measured mitochondrial protein levels and maximal citrate synthase (CS) activities in skeletal muscles.
Results: There was no significant difference in effect of two weeks endurance training between untrained mice and those that have experienced endurance training once. After two weeks training, the amount of NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 8 (NDUFB8) and Cytochrome c oxidase I (MTCO1) increased in the plantaris muscle regardless of previous trained or not. On the other hand, the detraining group, which rested for 4 weeks following endurance training, showed significant higher peroxisome proliferators-activated receptor-γ co-activator-1α(PGC-1α) and NDUFB8 contents in plantaris muscle than the control group.
Conclusion: The present study showed that the PGC1α and NDUFB8 in the detraining group was still higher than the control group although the detraining group had 4 weeks rest after training period. However, no clear difference in adaptation was observed between training group and retraining group following 2 weeks training.

Keywords: endurance training, mitochondria, retraining, detraining, muscle memory

Poster

P2-13

Transcriptional analysis of equine skeletal muscle following high-intensity interval exercise: A comparison of two different rest intervals

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It is well-known that exercise-induced gene expression depends on the intensity of exercise. Although high-intensity interval training is widely accepted as an effective strategy for skeletal muscle adaptation, the effect of duration of rest interval is not fully elucidated. In this study, we used Thoroughbred horses as an animal model of top athletes. In a randomized crossover design, eight Thoroughbred horses performed three 1-minute bouts of high-intensity exercise on a treadmill at the speed of their maximal oxygen uptake, with 15 minutes or 2 minutes of rest in between. Gluteus medius muscles were taken before and 4 hours after each exercise protocol. Peak plasma lactate concentrations were confirmed to be higher in the 2-minute rest condition than in the 15-minute rest condition. We first assessed the mRNA expression of several previously reported top exercise-responsive genes using quantitative real-time PCR. All of these genes were found to be upregulated by both exercises, with some genes, including *PPARGC1A*, being higher in the 2-minute rest interval protocol. Then, we further examined the mRNA expression of key transcription factors associated with mitochondrial biogenesis. However, except *PPARGC1A*, there was no significant difference between the two conditions in the magnitude of the mitochondria-related gene expression response to exercise. Although the proportion of ATP supply shifts from the glycolytic system towards oxidative metabolism as rest duration increases, our results indicate that two forms of exercise with different rest intervals induce similar initial adaptations in skeletal muscle, suggesting that the transcriptional response to acute high-intensity interval exercise occurs regardless of the duration of the rest periods.

Keywords: HIIT, skeletal muscle, PPARGC1A, lactate, Thoroughbred

P2-14

Aerobic exercise training improves learning and memory function and modulates neuroinflammatory responses in LPS-induced amnesic model

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Physical activity has been considered an important non-medication intervention to preserve mnemonic processes during aging. However, how aerobic exercise promotes such benefits for human health remains unclear. A possible hypothesis is that brain-metabolic changes of regions responsible for memory consolidation is affected by aerobic exericse training. The purposes of the present study we explore the acute effects of aerobic exercise training (AET) on neurocognitive performance and molecular biomarkers in lipopolysaccharide (LPS)-induced C57BL/6J mice, and to investigate the relationships of aerobic exercise-induced neurocognitive changes with changes in irisin and BDNF levels elicited by aerobic exercise models. In the *in vivo* study, eight-week-old C57BL/6J mice were randomly divided into four groups: group I; control, group II; LPS, group III; AET, and group IV; AET + LPS. Their AET were performed 10 rpm/min for 4 weeks after the intraperitoneal injection (0.1 mg/kg) of LPS. We found that AET ameliorated memory impairment and simultaneously improved memory function in the LPS-induced amnesic mice. Correspondingly, AET significantly increased the protein expression of neuroprotective markers, such as BDNF and CREB, upregulated Nrf2/HO-1 expression; and decreased the protein and mRNA expression of IFN-γ, IL-10, IL-1β, iNOS, COX-2, GCN5, and iba-1 in the hippocampus and cortex. Furthermore, AET boosting brain levels of FNDC5/Irisin and rescue the impaired special learning and cognitive function in LPS-induced C57BL/6J mice. In the *in vitro* study, Irisin treatment (12.5, 25, and 50 ng/mL) markedly attenuated the translocation of NF-κB to the nucleus concomitantly with the significant mitigation of the LPS-induced production of inflammatory mediators, including iNOS and COX-2. In addition, the Irisin treatment suppressed the phosphorylation of MAPK signaling and specifically inhibited the Phosphorylation IRF-3 in the LPS-stimulated BV-2 cells. By showing that FNDC5/irisin is an important mediator of the beneficial effects of exercise in mild cognitive impairment associated with AD model, our findings suggest that FNDC5/irisin as a novel agent capable of opposing neuroinflammation and memory impairment in AD.

Keywords: Aerobic Exercise, FNDC5/Irisin, neuroprotection, neuroinflammation, mild cognitive impairment

p2-15

Aerobic exercise with or without mask has positive effect on NGF and VEGF levels

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Decreased cognitive function and dementia are health problems in adults and the elder population. Nerve Growth Factor (NGF) is a protein that plays a significant role in the process of growth, development, and also maintenance of nerve cells. Vascular Endothelial Growth Factor (VEGF) is a protein that plays a role in angiogenesis, neuroprotection, neurogenesis and neuroplasticity. Regular aerobic physical exercise leads to an increase in the expression of serum NGF and VEGF, which affect nerve stimulation and cognitive function. The aim of the study was to analyze cognitive function in aerobic physical exercise with various types of masks based on NGF and VEGF levels. This study will benefit in terms of improving cognitive function and public health status. Experimental research was done with productive age participants at the Faculty of Medicine, Universitas Trisakti. The participants were divided into 3 groups, namely the control group, the cloth mask group and the medical mask group. Aerobic physical exercise was carried out 3 times per week for 4 weeks. Cognitive function was evaluated by measuring the levels of NGF and VEGF levels in all groups and there was no significant difference of means in all groups. However, there was a significant difference of NGF and VEGF level (p=0.000) from pre-exercise compared to post-exercise in the same group for all three groups. It can be concluded that aerobic physical exercise without a mask, with a cloth mask, or a medical mask for 4 weeks increases serum NGF and VEGF levels.

Keywords: aerobic exercise, cognitive function, mask, NGF, VEGF

P2-16

Different exercise intensities modify ACE2/MasR/ eNOS mRNA expression in Rat's lung

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Background: Exercise has various effect on organ function. In lung, exercise improve the lung vascular function through NO. NO production was induced by specific intensity duration. However, study of intensity duration on NO specific pathway is still limited. Thus, aim of this study was to analyze effect of different exercise duration on ACE2/MasR/ eNOS axis.
Methods: This study was used Male Wistar rats and divided into 4 different groups (control, low intensity, moderate intensity and high intensity exercise). Rats were trained using treadmill for 30 min/day, 5 times per week. Rats were terminated by the end of eight weeks training. Lungs were isolated and subjected to ACE2, MasR and eNOS mRNA expression examination. Morphology of lung's vascular wall were analyzed to determine the anatomical changes in subjects.
Results: We observed that high intensity exercise significantly increase the mRNA expression of ACE2, MasR, and eNOS ($p < 0.01$). In the other hand, Moderate-intensity exercise significantly increased MasR and eNOS mRNA expressions only ($p < 0.05$). Histological study showed that no change in medial wall thickness of lung arteries.
Conclusion: High-intensity exercise increase mRNA expression of ACE2, MasR, and eNOS, but without decreasing the medial wall thickness of the muscular artery.

Keywords: exercise, MasR, eNOS, ACE2

P2-17

Differences in thermographic response to aerobic and anaerobic exercise

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Background: Many studies investigated infrared (IR) thermographic changes following exercise mostly aerobic, but much less, following short, vigorous anaerobic exercise.
Aim: To investigate the surface temperature changes of the respiratory muscles (chest) during high intensity aerobic exercise and of the lower and upper limbs, following an anaerobic effort.
Methods: Thermal images were taken at rest, and then during each stage of the aerobic exercise (until exhaustion) from 18 volunteers, and immediately after the Wingate anaerobic test, from 24 volunteers. Images were processed to obtain a mean and max temperature in the regions of interest. We also developed an algorithm to calculate the distribution of temperature and texture (entropy) within each region.
Results: No changes were found in absolute temperatures, though the entropy of the chest surface area increased significantly throughout the aerobic exercise test, and was significantly correlated with exercise duration, intensity, and pulmonary ventilation ($p < 0.001$). Following the anaerobic exercise maximal surface temperatures were significantly higher in all measured regions ($p<0.04$). Participants who exhibited lower anaerobic capacity had a higher delta increase in surface leg's temperature compared with participants with higher anaerobic capacity who had a minimal change in leg's temperature.
Conclusion: Non-invasive thermal imaging during aerobic exercise showed high correlation with the work of breathing during high intensity aerobic exercise, while following anaerobic exercise, surface temperatures continue to increase even into the recovery period. Further studies are required to validate our results on other patient populations.

Keywords: aerobic exercise stress test, Wingate anaerobic test, thermographic images

Poster

P2-18

Effects of a 6-week aquarobic fitness program [AFT] in obese females during COVID-19

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Physical activity and exercise have been shown to have positive effects. We are designed to move, and when we regularly engage in physical activity, many of our body's systems function better. Aquarobic training is a type of aquatic aerobic exercise that mimics the aerobic dance pattern and arm movement pattern. There hasn't been much research on the effectiveness of aquarobic training programs because this practice isn't popular yet. The purpose of this study was to examine how a 6-week aquarobic training program [AFT] affected the physical fitness of obese females during COVID-19. The aquarobics training program [n = 15] and the control group [n = 15] each included 30 obese women as participants. For six weeks, the subjects were instructed to work out an aquarobics training program for 60 minutes a day, three times per week, at an intensity of 50 to 75% of their maximum heart rate. Data were collected on the body fat percentage, BMI, and hemoglobin levels before and after treatment, as well as the aquarobics training program intensity of 75% HRmax. Hypotheses are tested using the one-way Anova, Kruskal-Wallis, and mean difference tests (Tukey HSD and Mann-Whitney's). Participants' ages and caloric intake were not statistically different, but body fat percentage, BMI, and hemoglobin levels after exercise were (p 0.05). Body Fat Percentage [%BF] Aqua training program and control group pre-test [37.82 + 1.00; 36.48 + 1.05.] post-test [30.44 + 1.26; 36.03 + 1.16, p=0.000], BMI [kg/m^2] Pre-test [31.29+0.98; 31.72+1.13]; post-test [30.29+1.15; 31.86+0.093, p=0.000]. Haemoglobin [g/dl] levels pre-test [12.90+0.76; 12.97+1.11], pos-test [14.00+2.83; 12.97+1.18, p=0.001]. Body fat percentage, BMI, and hemoglobin levels in the aquaculture training group were significantly lower than in the control group. More research is needed, but the aquatic environment may offer some benefits for off-loaded exercise at high intensities in populations that are weak, injured, or in pain.

Keywords: aquajogging, waterfitness, physical fitness

P2-19

Developing and assessing the feasibility of a self-managed lifestyle intervention, as an adjunct therapy to compression for people with venous leg ulcers and early-stage neurodegenerative diseases

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Background: Neuro-degenerative disease (ND) is an umbrella term for conditions, which primarily affect the human brain neurons (i.e., dementias, Parkinson's disease). Among these, dementias represent over 70% of the cases. Thus, it is no surprise that almost 20% of those who receive home treatment for Venous Leg Ulcers (VLUs) have NDs as well. People with early-stage NDs appear to be either i) reluctant to participate in lifestyle interventions aiming to reduce healing times, or ii) aren't referred to these, despite the potential clinical benefits, because of their condition. Therefore, it is important to adapt support therapies to help them overcome the participation challenges faced by people with NDs.
Methods: We engaged with this VLUs sub-group (e.g., people with early-stage Alzheimer's or Parkinson's), who are ≥18 years' old, had VLU(s) of diameter ≥1 cm, ABPI≥ 0.8, and were able to tolerate lower-leg compression) and their carers, in a three-phased study. In Phase-1, we adapted our previously developed (FISCU-II) lifestyle intervention, comprising exercise and behaviour support, explore its feasibility and monitor their physiological, exercise and clinical progression. In Phase-2, we had two assessment days (pre- and post-intervention), collecting ulcer-related data, anthropometrics, baseline exercise and medical history, quality of life and physical fitness (via the Senior Fitness Test (2-min walking test, chair sit and stand) and ankle range of motion). Participants then completed a 4-week "training crash-course" (using a 3 sessions/week format – 12 sessions in total), among the participants that completed the interviews. In Phase-3, we carried out post-interviews, to explore the participants' study experiences.
Results: Nineteen people were approached; ten participants accepted to participate (53%) and seven were recruited, consented and interviewed. All assessments were completed successfully, with no exercise-related adverse events. All participants completed the 4-week intervention, however, only 20% of the sessions were independently-completed; the rest required support, by carers or the intervention facilitators. Participants enjoyed the interaction and found the study useful. Improvements were observed in all physical fitness fitness assessments (e.g., 2-min walking test; 13 (0-30) vs 17 (0-40), baseline vs 1-month respectively) and excellent ulcer healing indices as well (e.g., only one active ulcer at 1-month).
Conclusion: Our findings suggest that the adapted intervention is feasible, enjoyable and well-received, having the potential to bring clinical and physiological benefits to study participants.

Keywords: neurodegenerative diseases, venous leg ulcers, exercise, behaviour support

P2-20

Physical activity in older adults and affected risk factors

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Background: The benefits of high physical activity have been known. Physical activity can prevent diseases, maintain independence, and improve the quality of life in older adults. Therefore, it is recommended that older adults engage in moderate to high-intensity physical activity with sufficient duration. However, physical activity markedly declines in older age. Information about physical activity in older adults is limited. Physical activity in older adults might be associated with some possible risk factors. This study aimed to know older adults' physical activity in Indonesia and possible risk factors.
Methods: This cross-sectional study involves more than 31.439 older adults aged 60 years or older. Data was taken from the Indonesian Family Life Survey-5 (IFLS_%). Physical activity was assessed using The International Physical Activity Questionnaire Short Version (IPAQ-S7S).

Physical activity levels were classified into; high, moderate, and low. Independent variables as possible risk factors were age, gender, education, hypertension, diabetes mellitus, coronary heart disease, cholesterol level, knee arthritis, smoking, depression, and possible Dementia. All independent variables were dichotomous categorized. Chi-square was used to analyze the relationship between the level of physical activity with independent variables. The association between high physical activity and independent variables was analyzed using multivariate logistic regression with the stepwise backward method. Significance was set at $p<0.05$ and a 95% confidence interval.
Results: Older adults met recommended physical activity at the age of 60-65 was 43.9%, at the age of ≥65 was 50.4%, and overall was about 50%. Bivariate analysis indicates that the level of physical activity was correlated with age, gender, education, smoking, cholesterol, and other variables ($p<0.05$), except hypertension and possible Dementia ($p=0.119$). Multivariate analysis showed that age ≥65 y.o, female, high education, no smoking, high cholesterol, DM, stroke, no arthritis, and no depression had a higher possibility of lack of high physical activity ($p<0.05$).
Conclusion: Our findings indicate that high physical activity in older adults was affected by age, gender, education, smoking, high cholesterol, diabetes, stroke, arthritis, and depression.

Keywords: physical activity, older adults, the indonesian family life survey, physical activity levels, the quality of life

P2-21

Assessing the effect of regular aquatic exercise on micro- and macro-vascular physiology of older adults: a randomised-controlled trial (ACELA II study)

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Background: Cardiovascular disease (CVD) remains the number one death cause in the Western world, affecting also severely the quality of life (QoL) of those who suffer. Of particular interest among the high CVD-risk groups are older people, as cardiovascular (CV) ageing affects pathophysiological pathways also implicated in CVD development. Exercise may delay CV ageing, and consequently prevent the development of CVD in older people. The current literature presents conflicting evidence with some studies showing no improvement in CV parameters, while others suggest a positive effect on the intima-media thickness of carotid arteries, hemodynamic and biochemical markers. ACELA II aimed to assess the micro- and macro-vascular function in older people randomised to either regular aquatic exercise or no intervention for an 8-week period.
Methods: This was a single-centre, pragmatic, two-arm randomized controlled trial conducted in Sheffield, UK. Participants were invited at Sheffield Hallam University for two visits (i.e., baseline and 8-week follow up). Following the baseline assessments participants were randomised to either the aquatic or control groups. The assessments were consisted of anthropometrics and demographics, smoking and clinical history, as well as macro- and microvascular function tests as assessed by flow mediated dilation (using ultrasound) and laser doppler flowmetry (using thermal hyperaemia), respectively. The aquatic group performed an 8-week (2 to 3 sessions per week) aquatic training including swimming and water-based exercise classes.
Results: Participants' characteristics did not present any differences between groups both at baseline and follow up assessments. Following the exercise intervention, the exercise group significantly improved the macrovascular function (9.8 ± 4.2%, $p<0.001$) compared to the control group (4.6 ± 2.5%). Statistically significant microvascular improvements were also found for the aquatic group both raw (4.1 ± 0.9, $p<0.01$) and the percentages of cutaneous vascular conductance (3.2 ± 1.1%, $p<0.5$).
Conclusion: The regular aquatic exercise seems to benefit both the macro- and microvascular function in older adults when compared to no exercise (i.e., sedentary lifestyle). Some of the vascular parameters (e.g., initial peak and plateau) did not show a significant difference rather a tendency towards improvement for the aquatic group. Based on previous studies, a longer (i.e., ≥ 12 weeks intervention) may induce significant changes for all the vascular parameters.

Keywords: swimming, water-based exercise, vascular function, flow mediated dilation

P2-22

Exercise regulates NAD^+ in the hippocampus to prevent cognitive decline induced by physical inactivity

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Regular exercise is essential for maintaining physical health, as demonstrated through experimental results in both humans and animals. Numerous studies have reported that physical inactivity (PI) is a primary factor causing obesity, metabolic diseases, cognitive impairment, and depressive states in humans. However, an established method for examining the effect of PI on physiological, biochemical, and neuroscientific parameters in animals has yet to be reported.
Nicotinamide adenine dinucleotide (NAD^+) is an essential coenzyme involved in cellular bioenergetics. The key rate-limiting enzyme for the salvage pathway of NAD^+ biosynthesis is nicotinamide phosphoribosyltransferase (NAMPT), which regulates NAD^+ levels in various tissues. A decrease in NAMPT levels in the hippocampus has been associated with a decline in cognitive function. However, further studies are necessary to examine the relationship between cognitive function and NAD^+ levels.
In this study, we investigated the effect of PI on cognitive functions and depressive-like states in mice using a new housing cage (PI cage), and we hypothesized that exercise could improve results induced by PI. We found that exercise could increase NAD^+ levels in the brain by elevating NAMPT levels in the blood and preventing cognitive decline due to PI.
Using the nano-tag method, we initially compared the daily physical activity of mice housed in PI cages to those in standard cages. The mice housed in PI cages exhibited a decrease in physical activity levels by approximately 50% compared to those in standard cages. We also examined the effect of regular low-intensity exercise on cognitive functions and depressive states in mice housed in PI cages. Specifically, we employed 30 minutes of treadmill running at a speed of 5-15 m/min, three days per week. Our results showed that this exercise regimen prevented cognitive decline and the onset of a depressive-like state caused by PI. Lastly, we investigated whether low-intensity exercise increased blood NAMPT levels at different time intervals.
To examine the effect of low-intensity exercise on NAMPT levels, we measured increased NAMPT levels in the blood after one hour of exercise. These findings suggest that exercise increases NAMPT levels in the blood, which contributes to preventing cognitive function decline caused by PI. Furthermore, our novel PI model, utilizing the PI cage to house mice, could be useful for examining the effect of PI on cognitive

function and the onset of a depressive-like state.

Keywords: NAD^+, Physical inactivity, exercise, cognitive function

P2-23

Validity of queen's college step test in Indian sportspersons

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Background: Maximum oxygen consumption or VO_{2max} is internationally accepted as the best predictor of one's cardiorespiratory fitness. Direct estimation of VO_{2max} is difficult because of its complicated, exhaustive, laborious, time consuming and expensive experimental procedure involving well equipped laboratory. Thus, application of indirect protocols become inevitable in researches, especially in field studies where large number of samples need to be evaluated. Queen's college step test (QCT) is one such simple indirect procedure to predict VO_{2max}. Applicability of QCT in Indian population needs population specific validation since it was originally developed in Western population. Validity of QCT in Indian sedentary populations have been reported but its applicability in Indian sportspersons has not yet been tested. Therefore, the present study was aimed to enumerate the applicability of QCT in different categories of Indian sportspersons.
Methods: 761 sportspersons [soccer (male = 75, female = 70), hockey (male = 80, female = 80), volleyball (male = 90, female = 55), swimmer (male = 75, female = 60), boxer (male = 42, female = 39) cricketer (male = 60, female = 35)] were recruited in the study and randomly separated into study group (on which the procedure was tested and equation was modified) and confirmatory group (on which the modified equation was validated).Direct estimation of $VO_{2max}$was performed by graded incremental exercise on bicycle ergometer followed by expired gas analysis in Scholander micro gas analyzer. Indirect prediction of VO_{2max}(PVO_{2max})was performedby application of standard procedure of QCT. Student's paired t–test, Bland and Altman method of limit agreement analysis, correlation statistic and regression analysis were performed for the statistical treatment of the data.
Results: There was no significant difference between VO_{2max} and PVO_{2max} in the study group of male players. But female players of all the categories showed significant ($p<0.001$) variation between these two parameters. Depending on the existence of significant negative correlation between heart rate and VO_{2max} in all the female groups, regression equations were computed. Application of these regression norms in the confirmatory group depicted insignificant variation between VO_{2max} and PVO_{2max}. Bland and Altman's limit of agreement analysis depicted substantially small variation when group specific modified equation was applied in the confirmatory group.
Conclusion: It is concluded that original protocol of QCT is applicable in case of male Indian sportspersons. In case of female Indian sportspersons, the existing protocol of QCT is not a valid one. The group specific computed regression norms are therefore recommended for valid and precise prediction of VO_{2max} in the female sportsperson groups.

Keywords: VO_{2max}, QCT, Bland and Altman analysis, regression norm, sportspersons

P2-24

Effects of differential social contacts between two rats on their motivation for rotatory wheel exercise

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Notably, continuous exercise and sports are effective in improving physical and mental health and preventing disease; however, it is difficult to achieve. Factually, the World Health Organization (WHO) reported that physical inactivity is a major risk factor for noncommunicable diseases and death worldwide; yet, global estimates indicate that one in four adults and > 80% of adolescents do not engage in sufficient physical activity. A way of effectively maintaining and improving exercise motivation is to exercise with others who are highly motivated to exercise. However, the physiological mechanisms by which exercise motivation is transmitted by others remain unclear. In this study, we aimed to establish a behavioral experiment model that could help examine whether social interaction affects exercise motivation. By combining two wheeled cages, we developed a social wheel cage that can change the partition between two rats. Sixteen male Long-Evans rats (4 weeks old), in eight separate pairs, were kept in the social wheel cage for 4 weeks. The partition between the cages was replaced weekly, either with a wire-mesh (paired condition) or black acrylic panel (single condition). As locomotive activity indexes, the number of wheel rotations and vibration frequency were measured using an abdominally embedded Nanotag® implant. Cross-correlation analysis was conducted on the temporal relationship between the timing of exercise of two paired rats. The study revealed no significant changes in the wheel rotation and locomotive activity between the pair and single conditions. Interestingly, the variation in locomotion of individual rats tended to be smaller in the paired condition than in the single condition. Furthermore, cross-correlation analysis revealed higher synchronization of exercise onset timing between the two rats in the paired condition compared to the single condition ($p < 0.05$). These findings suggest that if one of the rats initiates exercise, the other's exercise motivation is triggered and synchronization of exercise motivation is induced. Overall, we developed a social wheel cage that can change the partition between two rats to establish a behavioral experiment model that could help examine whether social interaction affects exercise motivation.

Keywords: motivation, exercise, social wheel cage, cross-correlation analysis, rat

P2-25

Improvement of cardiopulmonary fitness by a yoga breathing exercise for seniors over 75- A pilot study conducted in superaged communities

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The American Heart Association considers cardiopulmonary fitness (CRF) an independent predictor of health and survival outcomes. Since CRF is an indicator of the integrated capacity of the heart and lungs to deliver oxygen to muscles during aerobic exercise, typical exercises increase heart rate (HR) up to 64-76% of the maximum heart rate (MHR) and improve CRF are running, swimming, and stair climbing. However, these exercises may not be suitable for the senior with poor dynamic ability. Since breathing training has been used in rehabilitation to improve CRF, it was feasible to explore whether the Sudarshan Kriya Yoga

breathing technique (SKY, a Yoga Breathing Exercise) could improve CRF in senior with poor dynamic balance. The senior over 75 years old in the superaged communities were invited to join the study. To accommodate the physical limitations of the senior, we have modified the technique for the safe sakes. Our Aim 1 was to determine the best way of measuring CRF in the senior after comparing cardiovascular parameters and dynamic ability between the young and the senior. Aim 2 was to compare exercise intensities of three tests to confirm the measurement of CRF in the senior. Aim 3 was to determine if a 6-month intervention with SKY improved CRF. Aim 4 was to reconfirm the positive effect of SKY on CRF after a 3-month intervention. Our results show that the ratio of HR to MHR in the senior (53.1±1.3%) is higher than that in the young (41±0.9%) ($p<0.05$). The time taken to complete the dynamic ability test in the senior was 9.5±0.6 seconds, which is higher than that of the young individuals (5.9±0.2 seconds). The HR of the senior increased to 63% of the MHR in the 2-minute step test (2-MST), 57% in the 30-second chair standing test, and 54% in the 30-second arm-curl test. After a 6-month intervention with SKY breathing, the step numbers in the 2-MST increased from 59±6 to 69±7 ($p<0.05$) (n=11). After discontinuing the intervention for 1 year, a 3-month intervention of SKY breathing was implemented again. The step number in the 2-MST increased from 55±4 to 65±5 ($p<0.05$) (n=9). Our data suggest that the SKY breathing technique effectively improves cardiopulmonary fitness (CRF) in the senior. However, the improvement ceases once the intervention is stopped.

Keywords: cardiopulmonary fitness (CRF), breathing exercise, Sudarshan Kriya Yoga (SKY), dynamic balance ability, senior

P2-26

Effect of sprint interval training on lipid profile in prediabetes

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Background: Prediabetes individuals have deranged glucose metabolism and are at an increased risk of progressing towards diabetes mellitus. Dyslipidaemia is frequently observed among individuals with prediabetes, and it is known to accelerate the process of atherosclerosis. Exercise is the lifestyle modification recommended for improving metabolic profiles in prediabetes subjects. However, lack of time is a common reason for non-adherence to exercise regimens. Sprint Interval Training is a modern and time-efficient exercise protocol which involves exercise at a higher intensity for shorter durations.
Objective: To evaluate the effect of sprint interval training on serum LDL, HDL, VLDL, and triglycerides in subjects with prediabetes.
Methods: Males aged 25 to 40 years with prediabetes, as defined by American Diabetes Association (ADA), i.e., fasting plasma glucose (100 to 125 mg/dL) or 2-hour plasma glucose (140 to 199 mg/dL); asymptomatic subjects with normal baseline electrocardiogram (ECG), and giving consent to participate in the study were enrolled. The 160 subjects included were randomly assigned to two groups: The Aerobic Exercise (AE) group, which did exercises at moderate intensity for 30 minutes per day and five days a week; Sprint Interval Training (SIT) group did exercise with an 'all-out' run effort for one minute that was followed by a recovery period of one and a half minutes completing a cycle of two and a half minutes. Four such cycles were performed in each session that required just 10 minutes a day. SIT was performed three times a week. Serum LDL, HDL, VLDL, and triglycerides were estimated before and after the exercise intervention period of 3 months.
Results: 72 subjects from the AE group and 74 from the SIT group completed the exercise protocols and the post-intervention evaluation. The mean age of the subjects in the AE group was 30.7 ± 3.3 years, and in the SIT group was 31 ± 3.4 years. There was a statistically significant improvement in serum LDL in the AE group from 161.6 ± 20.9 mg/dl to 153.7 ± 16.3 mg/dl ($p<0.001$) and in the SIT group from 164.4 ± 22.9 mg/dl to 155.1 ± 18.6 mg/dl ($p<0.001$). Similarly, there was an improvement in other lipid profile parameters after exercise in both groups, i.e., serum HDL from 39.1 ± 6.1 mg/dl to 40.2 ± 4.9 mg/dl in AE group and from 38.4 ± 6 mg/dl to 39.4 ± 6.3 mg/dl in SIT group; serum VLDL from 31.5 ± 5.5 mg/dl to 30 ± 5.7 mg/dl in AE group and from 32.1 ± 5.3 mg/dl to 29.7 ± 5.2 mg/dl in SIT group; and serum triglycerides from 169.3 ± 32.1 mg/dl to 159.8 ± 28.7 mg/dl in AE group and from 162 ± 21.9 mg/dl to 150.2 ± 19.9 mg/dl in SIT group.
Conclusion: Sprint Interval Training can be recommended as a practical and feasible protocol for improving lipid profiles in subjects with prediabetes. Sprint Interval Training can be particularly beneficial for individuals with less available time.

Keywords: aerobic exercise, serum LDL, triglycerides, time, glucose

P3-1

Chronic and acute druq-induced cardiotoxicity assessment using in vitro human iPSC-cardiomyocytes

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The Comprehensive in-vitro Proarrhythmia Assay (CiPA) paradigm was introduced to provide a more complete assessment of proarrhythmic risk by evaluating and implementing currently available high throughput methods. An important part of this is the electrophysiological evaluation of hERG, and also other cardiac channels including NaV1.5 and CaV1.2. The Q&A draft from August 2021 describes how nonclinical assays such as patch clamp can be used for integrated risk assessment prior to first-in-human studies, and in later stages of clinical development.
Taking into account the newly proposed ICH/S7B guidelines for cardiac safety, we characterized commercially available human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) to complement the assays available for cardiac safety testing. Since the ICHS7 guidelines do not address chronic treatment-induced changes in blood pressure, heart rate and/or repolarization, we assessed the effects of acute and prolonged exposure of reference agents known to cause functional changes in the heart, using in vitro tools employing hiPSC-CMs. That includes Anthracyclines, tyrosine kinase inhibitors and trafficking inhibitors that have been linked to changes in cardiac function over prolonged exposure in patients. We further combined automated patch clamp recordings with a contractility monitoring technology, focusing on a mature cardiac phenotype.
Here we studied the effects of calcium, sodium and hERG channel modulators at room temperature and at 37°C. Using hiPSC-CMs we show that late current (INa- Late), recorded during the ramp phase of the CiPA voltage protocol, was enhanced by ATX-II and blocked by ranolazine. Also mature physiological responses of hiPSC-CMs treated with positive inotropic substances such as L-type calcium channel agonist S-Bay K8644, beta- adrenergic agonist isoproterenol and cardiac myosin activator omecamtiv mecarbil are shown.
Our data demonstrate that cardiac ion channel pharmacology can be successfully recorded using hiPSC-CMs in various platforms, providing a reliable tool for cardiac safety screening and the study of cardiac ion

Poster

channel diseases in a model system closer to in vivo physiology than heterologous expression systems.

Keywords: CiPA, chronic, cardiac safety, contractility, electrophysiology

P3-2

Insulin signaling is critical for sinoatrial node maintenance and function

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Insulin and insulin-like growth factor 1 (IGF-1) signaling regulate cellular growth and glucose metabolism in the myocardium. However, their physiological role in the cells of the cardiac conduction system has never been explored. Therefore, we sought to determine the spatiotemporal function of insulin/IGF-1 receptor in the sinoatrial node (SAN). We generated cardiac conduction cell-specific inducible IGF-1 receptor (IGF-1R) knockout (KO) (CSIGF1RKO), insulin receptor (IR) KO (CSIRKO), and IR/IGF-1R double-KO (CSDIRKO) mice and evaluated their phenotypes. Telemetric electrocardiography revealed regular sinus rhythm in CSIGF1RKO mice, indicating that IGF-1R is dispensable for normal pacemaking. In contrast, CSIRKO and CSDIRKO mice exhibited profound sinus bradycardia. CSDIRKO mice showed typical sinus node dysfunction characterized by junctional rhythm and sinus pauses on electrocardiography. Interestingly, the lack of an insulin receptor in the SAN cells of CSIRKO and CSDIRKO mice caused sinus nodal fibrosis. Mechanistically, hyperpolarization-activated cyclic nucleotide-gated channel 4 (HCN4) protein expression significantly decreased in the CSIRKO and CSDIRKO mice relative to the controls. A patch-clamp study of the SAN cells of CSIRKO mice revealed a significant decrease in the funny current, which is responsible for spontaneous diastolic depolarization in the SAN. This result suggested that insulin receptor loss reduces the heart rate via downregulation of the HCN4 channel. Additionally, HCN1 expression was decreased in CSDIRKO mice, explaining their sinus node dysfunction. Our results reveal a previously unrecognized role of insulin/IGF-1 signaling in sinus node structural maintenance and pacemaker function.

Keywords: Insulin, Signaling, Sinoatrial node

P3-3

Requirement of β subunit for the reduced voltage-gated Na^+ current of a Brugada syndrome patient having novel double missense mutation (A385T/R504T) of *SCN5A*

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Brugada syndrome (BrS) a fatal arrhythmic disorder mainly associated with mutations in *SCN5A* encoding voltage-gated Na^+ channel α-subunit 5 ($Na_v1.5$). Here we identified a novel double missense mutation (A385T/R504T) of *SCN5A* in a patient with type 1 BrS electrocardiogram pattern. This study aims to elucidate the electrophysiological phenotype of A385T/R504T to understand the pathogenesis of the BrS patient. Whole-cell patch-clamp technique was used to analyze the $Na_v1.5$ current (I_{NaV}) in HEK293 cells transfected with the wild-type (WT) and mutant SCN5A with or without its β1-subunit (*SCN1B*) co-expression. The density of I_{NaV} was not altered in A385T/R504T $Na_v1.5$ alone. Furthermore, a rightward shift of the voltage-dependent inactivation and faster recovery from inactivation was observed, suggesting a gain-of-function state contrary to the BrS phenotype. Intriguingly, the co-expression of β1 with A385T/R504T revealed significant reduction of I_{NaV} and slower recovery from inactivation, consistent with the BrS phenotype. Notably, the total protein expression of *SCN5A* remained unchanged in A385T/R504T; however, the expression of membrane protein was reduced in A385T/R504T when co-expressed with β1. The β1-dependent reduction of I_{NaV} was observed in R504T while not in A385T single mutation. In contrast, the slower recovery from inactivation with β1 was observed in A385T while not in R504T. The expression of *SCN1B* is indispensable for the electrophysiological phenotype of BrS with the novel double mutations; A385T and R504T contributed to the slower recovery from inactivation and reduced levels of membrane expression as well as the current density of NaV1.5, respectively.

Keywords: SCN5A, SCN1B, sodium channel, missense mutation, Brugada syndrome

P3-4

Translation reinitiation in c.453delC frameshift mutation of *KCNH2* producing functional hERG K^+ channels with mild dominant negative effect in the heterozygote patient-derived iPSC cardiomyocytes

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The c.453delC (p.Thr152Profs*14) frameshift mutation in *KCNH2* is associated with an elevated risk of Long QT syndrome (LQTS) and fatal arrhythmia. Nevertheless, the loss-of-function mechanism underlying this mutation remains unexplored and necessitates an understanding of electrophysiology. To gain insight into the mechanism of the LQT phenotype, we conducted whole-cell patch-clamp and immunoblot assays, utilizing both a heterologous expression system and patient-derived induced pluripotent stem cell-cardiomyocytes (iPSC-CMs) with 453delC-*KCNH2*. We also explored the site of translational reinitiation by employing LC/MS mass spectrometry. Contrary to the previous assumption of early termination of translation, the findings of this study indicate that the 453delC-*KCNH2* leads to an N-terminally truncated hERG channel, a potential from a non-canonical start codon, with diminished expression and reduced current (I_{hERG}). The co-expression with wildtype *KCNH2* produced heteromeric hERG channel with mild dominant-negative effect. Additionally, the heterozygote patient-derived iPSC-CMs exhibited prolonged action potential duration and reduced I_{hERG}, which was ameliorated with the use of an hERG activator, PD-1180574.The results of our study offer novel insights into the mechanisms involved in congenital LQTS associated with the 453delC mutation of *KCNH2*. The mutant results in the formation of less functional N-terminal-truncated channels with reduced amount of membrane expression. An hERG activator is capable of correcting abnormalities in both the heterologous expression system and patient-derived iPSC-CMs.

Keywords: Long QT syndrome 2, KCNH2, frameshift mutation, translation reinitiation, iPSC-derived cardiomyocyte

Poster

P3-5

Distinct frequency-dependent alterations in local Ca^{2+} releases in left atrial versus right atrial myocytes under normal conditions and mechanical stimulus

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Ca^{2+} releases in the cell interior govern cell contractility, but their regulatory mechanisms in atrial myocytes, lacking transverse tubules, are poorly understood. Loss of atrial contractility is associated with atrial blood stasis and decrease of ventricular filling at diastole. The conditions such as atrial fibrillation can be caused by high hemodynamic disturbance including shear stress and rapid beating. AF indeed occurs more often in left atrial (LA)- than right atrial (RA)-chamber. We investigated if and how Ca^{2+} releases from the cell interior versus peripheral junctional Ca^{2+} releases are altered by increased beating rate and high fluid shear stress, and compared these responses between LA and RA myocytes. Rapid 2-D confocal Ca^{2+} imaging was used to simultaneously measure peripheral and non-junctional (central) Ca^{2+} releases in rat atrial myocytes. Shear stress was applied onto single cell by pressurized fluid-puffing. Acute increase of the rate of field-stimulation to 3 Hz from 1 Hz attenuated Ca^{2+} transients in LA- but not in RA-cells although diastolic [Ca^{2+}] increased in both sides. In addition, distinguished properties in the local Ca^{2+} changes in rapid stimulation includes faster Ca^{2+} decay in both RA and LA cells. Central release sites in LA cells showed distinctly faster release and decay than those of RA cells, and showed stronger deteriorations in their activities at higher stimulation even with unaltered SR Ca^{2+} content. Shear stress transiently enhances peripheral and central Ca^{2+} releases on depolarization with much higher stimulation on the central sites, which may enhance atrial contractility. High rate of depolarization removed this shear-induced strong Ca^{2+} release only in LA, not in RA cells. This could be explained by such distinct failure in depolarization-induced Ca^{2+} release in LA cell as well as its faster Ca^{2+} decay.

Keywords: left and right atrial cell, frequency-dependence, local Ca^{2+}, shear stress, Ca^{2+} decay

P3-6

A role of TRPM4 in slowing atrial autorhythmic Ca^{2+} cycling and its downregulation in failed atrial myocytes

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Transient receptor potential melastatin 4 (TRPM4) is a Ca^{2+}-activated monovalent cation channel and more abundant in atrial myocytes compared to ventricular myocytes. We have previously reported that TRPM4 is activated by Ca^{2+} releases initiated by inositol 1,4,5-trisphosphate receptor type 2 under prolonged fluid shear stress, and that it shows outwardly rectifying current-voltage relationship in isolated rat atrial myocytes. To know its function in atrial Ca^{2+} signaling and rhythm we compared the rate of rhythmic Ca^{2+} releases, and magnitudes of Ca^{2+} transients and caffeine-induced Ca^{2+} transients in HL-1 atrial cells transfected with TRPM4-specific siRNA and wild-type HL-1 cells using confocal Ca^{2+} imaging. In addition, TRPM4 current was detected using whole-cell patch clamp method in atrial cells isolated from sham-operated rats and rats with chronic (>20 weeks) transverse aortic constriction (TAC). We found that TRPM4 knock-down significantly accelerates the rate of Ca^{2+} cycling with no change in the magnitudes of Ca^{2+} transients and sarcoplasmic reticulum (SR) Ca^{2+} loading. To further examine the function of TRPM4 in intact atrial myocytes, we compared TRPM4 currents activated by shear stress. In left atrium (LA) cells from TAC rats showed significant hypertrophy compared with those from sham rats in terms of cell capacitance. Shear stress consistently enhanced ramp currents detected at voltage ramp from –120 mV to +70 mV, which was mostly suppressed by the TRPM4 blocker 9-phenanthrol (9-PT; 30-100 μM). The TRPM4-specific currents measured as 9-PT-sensitive currents were significantly decreased in failed LA cells from chronic TAC rats compared to those from sham. TRPM4 protein expression was consistently lower in TAC LA than sham LA. Our data suggest that TRPM4 is a negative regulator for atrial beating rate and SR Ca^{2+} loading. TRPM4, however, may not significantly affect regular depolarization-induced Ca^{2+} releases in atrial myocytes. In the failed left atrium induced by chronic pressure overload, shear-mediated TRPM4 activation is significantly attenuated, which may partly due to decreases in IP_3R2 and TRPM4 protein levels.

Keywords: TRPM4, atrial myocytes, HL-1 cells, heart rate, heart failure

P3-7

Modulation of transient outward potassium current by novel *SCN5A* mutants (p.A385T/R504T): implications for brugada syndrome phenotype

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Brugada syndrome (BrS) is an inherited cardiac channelopathy that significantly increases the risk of ventricular tachycardia and fibrillation, leading to sudden cardiac death. Characteristically, the BrS ECG shows ST-segment elevation primarily in the right precordial leads (V1-V3), which is attributed to either loss-of-function (LOF) variants affecting Na^+ current (I_{Na}) or gain-of-function (GOF) variants affecting transient outward currents (I_{to}). It was reported that the missense mutants p.A385T/R504T in *SCN5A* are associated with BrS. The decrease of I_{Na} required co-expression of the β-subunit *SCN1B* as well as A385T/R504T. To gain further insights into the mechanisms underlying the BrS phenotype, we employed whole-cell patch-clamp techniques using patient-derived induced pluripotent stem cell-cardiomyocytes (iPSC-CMs/BrS) and a heterologous expression system. Specifically, we co-transfected *SCN5A* with I_{to}-related genes (*KCNA4*, *KCND2*, *KCND3*) and the auxiliary subunit *KChIP2*, with or without *SCN1B*. The iPSC-CMs/BrS recapitulated the accentuated phase-1 notch in the action potential. Along with the reduced I_{Na}, notably, we observed an enhanced I_{to} compared to the healthy control iPSC-CMs. In the heterologous expression system, all the I_{to}-related currents were enhanced when co-expressed with *SCN5A*-A385T/R504T and *SCN1B*, but not without *SCN1B*. The NaV1.5 β1-dependent augmentation of I_{to} by p.A385T/R504T suggested multiple regulatory interactions with *KCNA4*, *KCND2*, and *KCND3*. It remains to be investigated whether there are reciprocal interactions between I_{Na} and I_{to}. These changes would be manifests as ST-segment elevation in the ECG and contribute to the development of the BrS phenotype.

Keywords: Brugada syndrome, SCN5A, transient outward current, arrhythmia

Poster

Poster

P3-8

Estrogen regulates transient outward potassium current through GPER under stress in hESC-CMs

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Background: Transient outward potassium current, in the phase 1 in action potential, plays a major role. The protective effect of estrogen on myocardium has been widely concerned. But it is unclear if estrogen regulate transient outward potassium channel under stress state. In this study, the effect of estrogen and its G protein-coupled estrogen receptor (GPER) on the transient outward potassium channel was observed under stress state.
Methods: The transient outward potassium current (I_{to}) of hESC-CMs was detected by Patch clamp technique; Immunofluorescence technology was used to detect the expression of Kv4.3 in hESC-CMs; Isoprenephrine (ISO, 10 μmol/L, 30 min) was used to make hESC-CMs stress models; G1 (11 nmol/L, 30 min) was used to stimulate GPER; G15 (20 nmol/L, 30 min) or small interfering RNA was used to block GPER.
Results: 1. Under non-stress conditions, administering estrogen prolonged the action potential duration at 10% repolarization (APD_{10}) and action potential duration at 20% repolarization (APD_{20}) of hESC-CMs. Estrogen had no effect on transient outward potassium current.
2. Estrogen pretreatment inhibited stress induced shortening of APD_{10}, APD_{20}, action potential duration at 90% repolarization (APD_{90}), and APD in hESC-CMs.
3. E2/G1 canceled the increase caused by stress in I_{to} of hESC-CMs.
4. E2 or G1 prevented the activation curve of transient outward potassium channel moving towards negative potential, the inactivation curve of transient outward potassium channel moving towards positive potential, and the recovery of the inactivation curve of transient outward potassium channel moving towards negative potential.
5. Inhibition of GPER counteracted the effects of E2 on I_{to} or transient outward potassium channels.
6. E2 had no effect on Kv4.3 expression under stressed or unstressed conditions.
Conclusion: Estrogen resists the increase in transient outward potassium current, the easily activation, an accelerated activation rate, a slower inactivation rate and an accelerated recovery rate after inactivation of transient outward potassium channel caused by stress in hESC-CMs through GPER. The effect of estrogen is not achieved by altering the expression of Kv4.3.

Keywords: estrogen, transient outward potassium current, hESC-CMs, G-protein coupled estrogen receptor, Kv4.3

P3-9

Insulin-resistance and nNOS signaling mediate fatty acid-induced inotropy and susceptibility of cardiac arrhythmias in hypertension

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The aim of the study is to evaluate the mechanisms mediating fatty acid (FA)-dependent pro-arrhythmic phenotype in angiotensin II-induced hypertensive (HTN) rat left ventricular (LV) myocytes. Metabolomics results showed that FA concentrations (oleic acid, linoleic acid, palmitic acid) were not different between sham and HTN rat arterial sera. Supplementation of these FAs and metabolic substrates including glucose, pyruvate and carnitine (named nutrient full solution, NF) increased LV myocyte contraction and intracellular Ca^{2+} transients ($[Ca^{2+}]_i$). Importantly, beta-adrenergic receptor stimulation with isoprenaline (ISO) in NF induced delayed-aftercontractions (DSCs) and spontaneous Ca^{2+} transients (sCa), and the frequency of DSCs was significantly higher in HTN. Electrophysiological experiments showed that action potential duration (APD) is shorter in HTN, and despite similar L-type Ca^{2+} current density between sham and HTN, Ca influx *via* I_{Ca} is greater and inward Na^{+}-Ca^{2+} exchanger current (I_{NCX}) was increased by NF+ISO in HTN. Accordingly, NCX inhibitor (KB-R7943) abolished DSCs, suggesting the role of $[Ca^{2+}]_i$. Immunoblotting experiments showed that tyrosine phosphorylations of insulin receptor (IR) and IR substrate-1 were blunted by NF in sham or in HTN. AKT-Ser^{473}/AKT and nNOS-Ser^{1417}/nNOS were increased by NF and the responses to insulin were lost in sham and HTN. However, NF+ISO increased AKT-Ser^{473}/AKT and nNOS-Ser^{1417}/nNOS only in sham but not in HTN and inhibition of nNOS with SMTC increased DSCs in sham but reduced DSCs in HTN. Intriguingly, SMTC increased both diastolic and systolic $[Ca^{2+}]_i$ in sham and reduced $[Ca^{2+}]_i$ in HTN. Our results indicate that increased $[Ca^{2+}]_i$ and NCX activity by NF+ISO mediates LV myocyte DSCs in HTN. Insulin receptor signaling is blunted by NF and nNOS activity plays pivotal roles in regulating $[Ca^{2+}]_i$ and in the promotion of arrhythmogenesis with FA.

Keywords: fatty acid, hypertension, arrhythmias, insulin-resistance

P3-10

Role of myofilament-associated nNOS for Ca^{2+} desensitization via troponin I phosphorylation in the right ventricular cardiomyocytes of rats

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This study aimed to compare the role of neuronal nitric oxide synthase (nNOS, NOS1) in the excitation-contraction coupling and myofilament Ca^{2+}-sensitivity between left ventricular cardiomyocytes (LVCMs) and right ventricular cardiomyocytes (RVCMs). Previous research had only investigated the effects of nNOS on LVCMs, but our study focused on RVCMs which have slightly larger diastolic sarcomere length (SL_D) and smaller sarcomere shortening (ΔSL) than LVCMs. Immunoblot study showed that the total expression and phosphorylated Ser^{1417} ratio of nNOS were not different between RVCMs and LVCMs. However, treating RVCMs with the nNOS-specific inhibitor, S-methyl-L-thiocitrulline (SMTC), resulted in more prominent augmentation of ΔSL and shortened SLD in RVCMs, along with a paradoxical decrease of the amplitude of Ca^{2+} transient ($\Delta[Ca^{2+}]_i$) due to a more significant inhibition of L-type Ca^{2+} current in RVCMs. The Ca^{2+}-contraction loop showed a more leftward shift in the RVCMs by nNOS inhibition, indicating Ca^{2+}desensitization of the myofilaments by the intrinsic nNOS. Previous studies offered Cardiac Troponin I (cTnI) phosphorylation as the regulatory target of myofilament Ca^{2+}-sensitivity through the sGC-cGMP-PKG pathway downstream to NO. The SMTC treatment decreased cTnI phosphorylation in the RVCMs, while not in the LVCMs, suggesting that nNOS plays a more prominent role in myofilament Ca^{2+} desensitization in RVCMs by targeting cTnI. Additionally, the higher phosphorylation of nNOSβ, the isoform of nNOS expressed in the myofilaments, was detected in the myofilament of RVCMs than LVCMs. This study is the first to demonstrate the more significant role of nNOS in RVCMs in regulating myofilament Ca^{2+} desensitization through cTnI.

Keywords: right ventricle, nNOS, myofilament, Ca^{2+} sensitivity, troponin I

P3-11

Deep learning-based simultaneous prediction of dose-response curves for ion channels

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Background: The Comprehensive In vitro Pro-arrhythmia Assay (CiPA) represents a new paradigm in the assessment of cardiac toxicity during the drug development process. In order to carry out an in-silico assay within the CiPA framework, it is essential to obtain a dose-response curve for each ion channel experimentally. As CiPA necessitates data from multiple ion channels, obtaining this information through the use of ion channels expressed in cell lines is the only viable option. Conducting experiments on each individual ion channel can be complex and time-consuming, and the inherent differences between ion channels expressed in cell lines and those found in natural cells can lead to errors in the experimental results. In an effort to mitigate these challenges, we aimed to explore the feasibility of simultaneously identifying dose-response curves for multiple ion channels in natural cells that contain the required channels.
Methods: To accomplish this, we developed a pulse protocol to generate current data that incorporates multiple ion currents. We utilized the O'Hara-Rudy (ORD) human ventricular cell model in conjunction with this protocol to generate 500,000 data. We employed a deep learning-based conductance prediction network, which we named the 'Block Prediction Network' (BPNet), and trained it using 300,000 data for training and 100,000 data for validation. The model was subsequently evaluated using a dataset of 100,000 data.
Results & Discussion: The BPNet was able to identify conductance with a high degree of accuracy, as evidenced by a correlation coefficient of 0.999 for seven ion channels. Subsequently, we generated current data for each drug by utilizing the dose-response data for 12 drugs published in CiPA for testing purposes. Our BPNet was able to identify the same dose-response for each ion channel. Our approach has two key advantages. One of which is that drug effect identification can be significantly accelerated as a result of this method. As an illustration, traditional methods would require 140 experiments (5 repetitions at 4 different concentrations for each of the 7 ion channels) to be performed. In contrast, our method only necessitates 20 experiments in natural cells, and thus, significantly reduces the number of experiments required by a factor of 1/(the number of ion channels). Additionally, we discovered that our approach allows for the identification of drug effects on the late sodium current without the need for ATX II (anemone toxin II) treatment.
Conclusion: While our method requires validation through experimental verification, it has clearly demonstrated the potential to decrease time, effort, and costs, as well as to transform the paradigm of new drug development.

Keywords: deep-learning, dose-response curve, CiPA, cardiac electrophysiology, multi-ion channel assay

P3-12

Propagation of repolarization in human ventricle revealed in a one-dimensional array of cardiomyocyte model

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Repolarization has been suggested to propagate in cardiac tissue theoretically as well as experimentally. However, it has been challenging to estimate how and to what extent the propagation of repolarization contributes to the relaxation of the heart because repolarization occurs spontaneously without propagation in each cell as is clear from experiments on isolated cardiac myocytes. Thresholds for all-or-nothing repolarization in multicellular preparations such as Purkinje fiber and papillary muscle fiber used to be one of the foci of the dispute. To investigate how the propagation of repolarization takes place in multicellular settings, we developed a 1-D array of the human ventricular cell model by connecting the cells electrically via gap junction channels and analyzed the ionic mechanisms underlying the repolarization in each cell in the array. Aiming at separating the propagation of repolarization from the natural course of spontaneous repolarization, we first established an array stabilized at a quasi-equilibrium potential near the plateau potential level by fixing the state probability of inactivation of the late sodium channel (I_{NaL}) at a low level and then applied a hyperpolarizing stimulus at the center of the cell array. Indeed, the repolarization induced at the center of the array propagated towards both ends of the array at a velocity of 1.1 cm/s, which was much slower than a velocity for excitation in the same array (43 cm/s). The level of fixed state probability of inactivation of I_{NaL} as well as the size of the inward rectifier potassium channel (I_{K1}) affected the velocity of the propagation. It was found that the ionic mechanisms responsible for the repolarization at the cell level, i.e. deactivation of I_{NaL} and the L-type calcium channel (I_{CaL}) and activation of the rapid component of delayed rectifier potassium channel (I_{Kr}) and I_{K1}, were also important for the propagation of repolarization in the cell array. Intercellular currents through gap junction channels were found to play an important role in supplying hyperpolarizing current large enough to overcome the increase in inward currents in response to the initial repolarization at the repolarization wavefront. In analogy to progressive activation of the transient sodium channel (I_{NaT}) at excitation, regenerative activation of I_{K1} was dominant in the later phase of repolarization. Considering the heterogeneities of the cells and complexities in the electrophysiology of the heart especially in pathophysiological conditions, it was suggested that the mechanisms of the propagation of repolarization should be understood quantitatively based on mathematical models.

Keywords: repolarization propagation, mathematical model, ventricle

P3-13

Evaluation of customized stenting in patients with atherosclerosis

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Background: Atherosclerosis is a disease in which lipid such as cholesterol accumulates in blood vessels and narrows or occlude blood vessels. Early diagnosis is essential because it does not cause symptoms

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until more than 70% of the arteries are stenosed. Percutaneous coronary intervention (PCI) is a typical method of treating atherosclerosis. However, PCI can cause recurrent stenosis in arteries due to Intimal hyperplasia (IH), in which new tissues grow excessively in the treatment area. Therefore, this study aims to analyze the effect of stents on coronary arteries through hemodynamic simulation and establish a system for predicting side effects that may occur after PCI.
Methods: Coronary arteries with ischemic heart disease were segmented based on Hounsfield units using CT images. After designing a 3d stent and inserting it into a blood vessel, the strain of the blood vessel was calculated, and the blood flow simulation was conducted. Hemodynamic parameters are analyzed using Computational fluid dynamics (CFD) and fluid-structure interaction (FSI). Various parameters based on WSS were obtained and analyzed.
Results: The time average wall shear stress (TAWSS), vibration shear index (OSI), and relative residence time (RRT) are operators based on the WSS. These parameters are well known as a significant indicator related to plaque production and rupture, and such hemodynamic parameters play an essential role in the progression of arteriosclerosis. Hemodynamic parameters obtained through simulation using CFD and FSI were comprehensively analyzed. Significant results were obtained when comparing stenosed blood vessels with those reconstructed by PCI. This result shows that the effect of stents on blood vessels can be evaluated using simulations.
Conclusion: Through the above results, the prognosis of PCI could be evaluated by simulation using CFD and FSI. Simulations are expected to predict the side effects of stents in advance and design optimal stent procedures in a non-invasive method. It is also expected to provide a diagnostic technique to quantify complex vascular physiology and predict an effective prognosis.

Keywords: coronary artery, computational fluid dynamics (CFD), fluid structure interaction (FSI), customized stenting, simulation

P3-14

S-Allylcysteine limits cardiac remodelling via antioxidative mechanism in estrogen-deficient rats subjected to myocardial injury

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Background: The incidence of cardiovascular diseases increases among post-menopausal women. *S-Allylcysteine* (SAC), one of the aged garlic extract compounds, has shown its cardioprotective effect through mitochondrial preservation and (cystathionine γ-lyase) CSE/hydrogen sulfide pathway (H_2S). Hence, this study was aimed to investigate the effect of SAC in estrogen-deficient rats subjected to myocardial injury (MI).
Methods: Female Wistar rats (n=40, 200-250 g) were used in this study. Ovariectomy surgery (OVX) was done onto 32 rats to suppress the estrogen levels, whereas eight rats were treated as Sham. After three weeks of recovery, all rats received administration of either normal saline (Sham, OVX; sq.) or isoprenaline 85 mg/kg (OVXIM, 2x; sq.) to induce MI. Then, oral gavage of either distilled water (Sham, OVX, OVXIM) or SAC 100 mg/kg (OVXS, OVXIMS) were given to the rats for one week. The rats were anesthetized with KTX (1 ml/kg, i.v.) before the hearts were excised and cannulated on Langendorff isolated heart system for cardiac function, and then stored for further biochemical and histological analysis.
Results and Discussion: The rat models were validated by decreased estrogen level, while MI was from the increased ST elevation on ECG and plasma Troponin T level. SBP in OVXS showed significant decrease by 15% after receiving SAC ($p<0.05$ vs. Sham). SAC did not reduce oxidative stress product TBARS in OVXIM; however, the treatment increased antioxidant GSH level ~55% ($p<0.05$, OVX vs. OVXMIS) and SOD antioxidants ~30% ($p<0.05$, OVX vs. OVXMIS). This could be due to allyl and cysteine, which both have antioxidative properties, protected the vascular thus reducing blood pressure. Histological observation showed that SAC treatment was able to limit hypertrophy and collagen deposition in OVXIM group significantly. This suggests that SAC antioxidant action may be able to limit the cell injury from oxidative stress, thus decreasing fibrosis effect ($p<0.05$, OVXIM vs. OVXIMS) and preserving cardiomyocyte size ($p<0.05$, OVXIM vs. OVXIMS). However, the treated rats showed unaltered cardiac function. CSE enzyme activity tended to return to the baseline and the CSE protein expression showed an increased pattern in OVXS as compared to Sham.
Conclusion: This study showed SAC's potential in limiting cardiac remodelling in estrogen-deficient condition, however further study is warranted to further understand antioxidant and CSE enzyme signalling pathway.

Keywords: S-Allylcysteine, ovariectomized, isoprenaline, CSE, antioxidant

P3-15

Impairment in pacemaker function of sinoatrial nodal cells in a mouse model of myocardial steatosis

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Background: Patients with diabetes mellitus and obesity have a high prevalence of sinus node dysfunction (SND). These patients often exhibit accumulation of lipid droplets (LDs) in cardiomyocytes; myocardial steatosis. However, the causal association between SND and myocardial steatosis has not been elucidated. Transgenic (Tg) mice with cardiac-specific overexpression of perilipin 2 (PLIN2), a mouse model of myocardial steatosis, exhibited accumulation of LDs around mitochondria in atrial myocytes (Sato et al., AJP-EM, 2019). We speculated that such accumulation of LDs in sinoatrial nodal cells (SNCs) could disrupt pacemaker activity based on our previous finding that the Ca^{2+}-mediated interplay between mitochondria, sarcoplasmic reticulum (SR), and channels/transporters at the plasma membrane contributes to automaticity of SNCs. We therefore investigated cardiac pacemaker function of PLIN2-Tg, in comparison to wild-type (WT) mice.
Methods: Electron micrographs (EM), electrocardiogram (ECG), reactive oxygen species (ROS), local calcium release (LCR) from SR and Ca^{2+} transient were compared between the SA node of WT and PLIN2-Tg mice.
Results: EM revealed accumulation of LDs, especially around mitochondria, and also partial swelling of SR in SNCs. ECG demonstrated increased R-R variability in PLIN2-Tg compared to WT mice, suggesting SND. Cytosolic and mitochondrial ROS levels were increased in PLIN2-Tg SNCs. The amplitude and size of LCR were reduced and the occurrence of early LCR tended to increase in PLIN2-Tg SNCs. The duration of Ca^{2+} transient was shortened whereas the variability of Ca^{2+} transient interval was increased in PLIN2-Tg SNCs.
Conclusion: These findings suggested that myocardial steatosis induces sinoatrial node dysfunction, which is associated with increased

mitochondrial ROS levels and impaired SR Ca^{2+} handling.

Keywords: myocardial steatosis, sinoatrial nodal cells, SR Ca^{2+} handling, lipid droplets

P3-16

CRBN^{cKO} leads to age-dependent cardiac fibrosis and senescence via unbalanced lipid metabolism and AMPK hyperactivation

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Cereblon (CRBN), a substrate receptor of the E3 ubiquitin ligase complex, has been known to regulate AMPK activation negatively. Previous studies showed that cardiac-specific CRBN knockout (CRBN^{cKO}) mice in 8 weeks increase cardiac contractility by regulating calcium signaling. However, it is unclear whether sustained cardiac-specific depletion of CRBN continues to be a beneficial effect. In the present study, we analyzed cardiac-specific CRBN knockout mice(αMHC-Cre+/-; CRBNflox/flox) in 37 weeks. The results demonstrated that CRBN^{cKO} mice in 37 weeks showed cardiac hypertrophy and systolic dysfunction in the heart. Notably, CRBN^{cKO} mice in 37 weeks induce alterations in lipid metabolism by AMPK hyperactivation in the heart. Subsequently, it caused excessive ROS production thereby impairing mitochondrial function. Moreover, we revealed that sustained CRBN^{cKO} in 37 weeks mice increased cardiac senescence and fibrosis. In conclusion, we suggest that CRBN is essential for metabolic homeostasis in the heart.

Keywords: cereblon, heart failure, senescence, fibrosis, lipid metabolism

P3-17

Evaluation of global and segmental strain in ApoE and APE1/Ref-1 double knockout mice using cardiac magnetic resonance imaging

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Background: Mouse models lacking Apolipoprotein E (ApoE) gene and fed a high-fat diet have been instrumental in studying atherosclerosis. However, these models do not accurately mimic inflammation-induced atherosclerosis. Apurinic/apyrimidinic-endonuclease 1/Redox factor 1 (Ref-1) is a multi-functional protein involved in DNA repair and regulation of oxidative stress and nitric oxide production, and its association with atherosclerosis has been observed in previous studies. This study is a baseline study to establish a more representative mouse model for studying the relationship between Ref-1 and inflammatory atherosclerosis. This study investigated the functional parameters and global and segmental strain of the heart in ApoE and Ref-1 double knockout (DKO) mice and ApoE single knockout (SKO) using preclinical cardiac magnetic resonance imaging (CMR).
Methods: We crossbred ApoE knockout mice with heterozygous Ref-1 mice with low expression of Ref-1, producing ApoE/Ref-1 DKO mice. ApoE/Ref-1 DKO mice (n=4) and ApoE SKO mice (n=2) aged 8-9 weeks on normal diet were included in this study. CMR using a 7T scanner and retrospective-gated GRE sequence was performed to assess the functional parameters of the left ventricle (LV) and right ventricle (RV). Global and segmental strain analysis of LV and RV was conducted. The non-parametric Mann–Whitney U test was used for group comparisons.
Results: The LV ejection fraction of ApoE/Ref-1 DKO mice was 47.5±3.78% and 56.5±0.70% (p=0.133) in SKO. The RV ejection fraction was 45.25±2.50% and 63.5±3.53% in SKO (p=0.133). Other LV and RV volumetric parameters were similar between the two groups. Global longitudinal strain (GLS) of ApoE/Ref-1 DKO mice was -0.21 ± 0.01 and -0.19 ± 0.03 in SKO (p=0.533). Global radial strain (GRS) of ApoE/Ref-1 DKO mice was 0.23 ±0.04 and 0.26 ±0.04 in SKO (p=0.533). Global circumferential strain (GCS) of ApoE/Ref-1 DKO mice was -0.18 ±0.01 and -0.20±0.02 in SKO (p>0.999). RV GLS of ApoE/Ref-1 DKO mice was -0.40±0.14 and -0.24±0.01 in SKO (p=0.533). RV GCS of ApoE/Ref-1 DKO mice was -0.29±0.02 and -0.33±0.00 in SKO (p=0.133). Global and segmental strain values were not significantly different in ApoE/Ref-1 DKO mice compared to SKO mice.
Conclusion: This study quantified volume and function indices of LV and RV. GLS, GRS, and GCS of LV were similar between ApoE/Ref-1 DKO mice and ApoE SKO mice. Volumetric and strain indices of mouse CMR images are reproducible parameters for cardiovascular research in transgenic mice models.

Keywords: ApoE, APE1/Ref-1, atherosclerosis, strain, CMR

P3-18

Carvacrol ameliorates cardiac remodelling in doxorubicin-induced cardiotoxicity rat model

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The phenolic monoterpenoid carvacrol, yielded by aromatic plants, has been demonstrated to have a cardioprotective effect by directly scavenging free radicals and improving heart function. Therefore, we aimed to explore the potential of carvacrol on cardiac hypertrophy and fibrosis in the doxorubicin-induced cardiotoxicity rat model.
Twenty-four male Sprague-Dawley rats (200-250g) were randomly divided into three groups; control, cardiotoxicity (DOX) and treatment groups (CAR+DOX). The control and DOX groups were given corn oil orally as vehicle control; meanwhile, carvacrol (50 mg/kg, BW) was given orally to the CAR+DOX groups for 14 days. Then, a single dose of doxorubicin (15 mg/kg/i.p, BW) was administered on day-15 for DOX and CAR+DOX groups; meanwhile, 0.5% of DMSO was given to the control group. The rats were left to recover for 3 days before sacrificing with KTX overdose, followed by heart removal. Throughout this study, systolic blood pressure (SBP) measurements were obtained via the non-invasive tail-cuff technique. Heart function was accessed by the Langendorff technique. Cardiac fibrosis was assessed by analyzing collagen accumulation with picrosirius red staining. Meanwhile, myocyte hypertrophy was observed from the myocardium size stained with haematoxylin and eosin staining. The cross-sectional area quantifications

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for both stainings were analyzed using the Image J software. Another experiment subset was conducted, and the heart samples were stored for further biochemical analysis.
On day-18 of the experiment, the results showed that SBP decreased significantly in the DOX group ($p<0.05$ vs control), indicating possible cardiac damage. Carvacrol alleviated the SBP, similar to the control group. However, the heart function remains unaltered in the carvacrol-treated group. There was a significant aggravation of cardiac fibrosis in the DOX group ($p<0.05$ vs control). These data were further supported by H&E staining that showed a significant difference between the DOX group ($p<0.05$ vs control), indicating myocyte hypertrophy in the DOX group by 13.4% compared to the control group. Along with treatment, the DOX group exhibited significant ($p<0.05$ vs CAR+DOX) myocyte hypertrophy decrement in CAR+DOX by 8.1%. Carvacrol treatment did not significantly change the oxidative stress marker (MDA) and antioxidant status (SOD, GSH) in all groups.
The results support the notion that carvacrol could be a constructive adjuvant to reduce cardiotoxicity in part through cardiac remodelling, particularly by ameliorating myocardial fibrosis and hypertrophy.

Keywords: cardiotoxicity, heart failure, cardiac remodelling, doxorubicin, carvacrol

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P3-19

Reduced TRPA1 expression in MCF improves TGFβ1-induced cardiac fibrosis

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Background: Transient receptor potential Ankyrin 1 (TRPA1) is a non-selective cation channel primarily studied for its nociceptive role in neurons. However, more recent studies confirmed its expression in other tissues such as cardiac tissues where it facilitates ion transport. This study aimed (1) to evaluate the regulation of TRPA1; and (2) to determine the role of TRPA1 in the physiology of cardiac fibrosis in vitro.
Methods: Western blot was performed to check the TRPA1 protein levels in vitro (mouse cardiac fibroblasts) fibrotic model. RT-PCR analysis was used to measure gene expression while immunocytochemistry was used to visualize the protein expression in cells. To create an *in vitro* fibrotic model, mouse cardiac fibroblasts (MCF) were treated with rhTGFβ1 (5 ng/mL). On the other hand, to create a TRPA1 knockdown model, siRNA targeting TRPA1 was treated to the cells.
Results: TRPA1 levels in MCF were found to increase under fibrotic conditions. Meanwhile, knocking down TRPA1 using siRNA decreased the expression of fibrotic markers, α-SMA and col1a1. Accordingly, the decreased levels of TRPA1 under fibrotic conditions attenuated the phosphorylation of SMAD2 and ERK1/2 proteins which led to decreased fibrotic markers.
Conclusion: The findings of this study showed that inhibition of TRPA1 is a potential strategy in addressing against TGF-β1-induced cardiac fibrosis. The study also provided evidence that TRPA1 affects the progression of fibrosis through the fibrotic ERK1/2 signaling pathway.

Keywords: TRPA1, TGFβ1, cardiac fibrosis

P3-20

Regulation of L-type voltage-dependent Ca^{2+} channel by cereblon

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Cereblon (CRBN) is a substrate receptor of the E3 ubiquitin ligase complex, which has been reported to target ion channel proteins. Modulation of the L-type voltage-dependent Ca^{2+} channel α1c subunit ($Ca_v1.2\alpha$) is a critical player in heart failure associated with reduced ejection fraction (HFrEF), but the underlying cellular mechanisms are unclear. Here, we explored the role of CRBN in HFrEF by investigating the direct regulatory role of CRBN in $Ca_v1.2\alpha$ activity, and how it can serve as a target to address myocardial dysfunction. Cardiac tissues from HFrEF patients exhibit increased levels of CRBN, suggesting its involvement in heart failure (HF). In vivo and ex vivo animal model studies demonstrate that whole body CRBN-knockout (CRBN-/-) and cardiac-specific knockout mice (Crbnfl/fl/Myh6Cre+,) enhanced cardiac contractility with increased L-type calcium channel current (ICaL), modulated by the direct interaction of CRBN with $Ca_v1.2\alpha$ subunit. Mechanistically, we discovered that the Lon domain of CRBN directly interacts with the N-terminal of $Ca_v1.2\alpha$. Increasing CRBN levels enhanced ubiquitination and proteasomal degradation of $Ca_v1.2\alpha$ and decreased L-type Ca^{2+} channel currents. By contrast, genetic or pharmacological depletion of CRBN via TD-165, a novel CRBN-targeting degrader, increased the surface expression of $Ca_v1.2\alpha$ and enhanced L-type Ca^{2+} channel currents. We have also discovered that CRBN-/- mice exhibited resistance to doxorubicin-induced HF. findings indicate that CRBN selectively degrades $Ca_v1.2\alpha$, which in turn facilitates cardiac dysfunction, suggesting that reducing CRBN levels could serve as a new target of cardioprotective therapeutic strategy for HF.

Keywords: cereblon, HFrEF, L-type voltage-dependent Ca^{2+} channel, cardiac contractility

P3-21

SGLT2 inhibition with empagliflozin ameliorates myocardial mitochondrial dysfunction in diabetic mice heart

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Diabetic cardiomyopathy (DCM) is one of the major causes of end-stage heart failure (HF), which contributes to mortality and morbidity in type 2 diabetes. Empagliflozin (EMPA), a sodium-glucose cotransporter 2 inhibitor (SGLT2i), showed cardioprotective effects against DCM but its molecular mechanism remains unclear. We investigated whether EMPA prevents heart dysfunction by reducing cardiac fibrosis and mitochondrial dysfunction in obese db/db mice. Five-week-old diabetic and obese db/db mice were treated with EMPA (10mg/kg/day) for 10 weeks via oral gavage, while db/db control mice and wild-type (WT) were received an equal amount of the vehicle. Results showed that Treated db/db mice with EMPA reduced body weight and blood glucose levels. Both systolic and diastolic functions of db/db mice were improved by EMPA in echocardiography analysis. In addition, EMPA attenuated myocardial hypertrophy and cardiac fibrosis in db/db mice. Moverover, EMPA treatment improved mitochondrial function by activating NRF2/

OXPHOS signaling pathway. Collectively, these findings demonstrate that EMPA prevents DCM by reducing cardiac fibrosis and mitochondrial dysfunction in type 2 diabetes mice.

Keywords: diabetic cardiomyopathy, SGLT2 inhibitor, empagliflozin, mitochondrial dysfunction

P3-22

Arachidonic acid-induced lipid peroxidation causes endosomal rupture in cardiac myoblasts

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Ischemia-reperfusion of the heart results in the accumulation of arachidonic acid (AA), which is an essential polyunsaturated fatty acid and plays a vital role in the inflammatory process, production of reactive oxygen species (ROS), and lipid peroxidation associated with ferroptosis in the plasma membrane. It significantly contributes to the cardiomyocytes injury caused by ischemia-reperfusion. Lipid peroxidation of biological membranes modifies their assembly, structure, and dynamics. Endosomes are primarily intracellular sorting organelles. It is widely believed that an endosome-dependent pathway of biogenesis generates exosomes. The aim of this study was to examine whether AA can trigger lipid peroxidation and endosome production to cause cardiomyocyte cell death. H9c2 myoblasts, flow cytometry, confocal microscopy, and MTT assay were used to investigate the correlation between AA-induced cardiomyocyte lipid peroxidation, endosome formation, and cell death. The endosome was labeled with SytoRNA and PKH26. Our results showed that AA could increase ROS and lipid peroxidation and reduce the cell viability in H9c2 myoblasts. In addition, treatment with AA significantly reduced the number of endosomes in the cells These effects were blocked by lipid ROS scavengers, such as ferrostatin-1, liproxstatin-1, and phenanthroline. In conclusion, the findings from the present study suggest that the AA-induced lipid peroxidation contributed to endosomal rupture and subsequent cellular damage in cardiac myoblasts. However, more studies still required for delineating the detailed mechanism.

Keywords: arachidonic acid, endosome, ROS, lipid peroxidation

P3-23

Parkin suppresses cardiomyocyte ferroptosis induced by iron overload through the ubiquitination of ACSL4 and the regulation of lipid metabolism

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Background: Iron overload is strongly associated with cardiovascular diseases. Iron overload induced ferroptosis is a new cell death pathway indicated in cardiac ischemia reperfusion injury. However, the precise molecular mechanism underlying ferroptosis in cardiomyocytes remains unclear. Parkin, an E3 ubiquitin ligase, has been shown to be closely linked to several cardiovascular diseases and plays a crucial role in cardiac function by mediating ubiquitination. This study aimed to investigate the role and molecular mechanism of Parkin in iron overload-induced ferroptosis in cardiomyocytes.
Methods: The role of Parkin in the heart was assessed using cardiac Parkin-specific knockout mice (*Myh6-Cre/Parkin*$^{f/f}$) and the rat heart cell line H9c2 treated with ferric citrate (FAC) to establish animal models of iron overload and cellular iron overload. Cell death was determined using PI staining, while lipid peroxidation was detected using MDA staining to confirm the occurrence of ferroptosis in cardiomyocytes induced by iron overload. Immunoprecipitation (IP) was employed to identify ACSL4 as an interacting protein of Parkin. Lipidomic analysis was conducted to determine the impact of iron on the levels of polyunsaturated fatty acids. Additionally, Parkin, ACSL4, and p53 were overexpressed and knocked down to verify their biological functions. Hypoxia-reoxygenation (H/R) and ischemia-reperfusion injury (I/R) models were employed to investigate the involvement of iron in these processes. Cardiac function and myocardial remodeling were evaluated using ultrasound and histological staining in small animals.
Results: We demonstrated that ferroptosis was the primary form of cell death in models of iron overload and I/R-induced cardiomyopathy. Mechanistically, we discovered that Parkin inhibited iron overload-induced ferroptosis in cardiomyocytes by promoting the ubiquitination of long-chain acyl-CoA synthetase 4 (ACSL4), a critical protein involved in ferroptosis-related lipid metabolism pathways. Furthermore, we identified p53 as a transcription factor functioning upstream of Parkin, which transcriptionally suppressed Parkin expression in iron overload cardiomyocytes and thereby regulated iron overload-induced ferroptosis. In animal studies, cardiac Parkin-specific knockout mice (*Myh6-Cre/Parkin*$^{f/f}$) fed a high-iron diet exhibited more severe myocardial damage, and high iron levels were found to exacerbate I/R injury. However, treatment with Fer-1, a ferroptosis inhibitor, significantly inhibited iron overload-induced ferroptosis and myocardial I/R injury. Moreover, overexpression of Parkin effectively prevented impaired mitochondrial function and mitochondrial lipid peroxidation induced by iron overload.
Conclusion: These findings reveal a novel anti-ferroptosis regulatory pathway involving p53-Parkin-ACSL4, providing a new perspective for the prevention and treatment of heart diseases.

Keywords: iron overload, Ferroptosis, Parkin, ACSL4, p53

P3-24

The roles of mitochondrial circFBXO25 in cardiac ischemia/reperfusion injury

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Background: Mitochondria can not only produce energy, but also regulate the life activities of cells by inducing apoptosis and necrosis, which is particularly important for heart disease. In fact, mitochondria can provide 90% ATP energy for cardiomyocytes, while the mitochondria pathway induced cell death is the main pathogenic factors of many cardiovascular diseases. In recent years, several studies have shown that circular RNAs (circRNAs) exist in mitochondria and plays an important role in regulating mitochondrial homeostasis. However, there are still few reports about nuclear-encoded circRNA which located in mitochondria, yet its function and transport mechanism are still unknown.
Methods: The circRNAs located in cardiac mitochondria were detected and identified by high-throughput sequencing, and then the expression of mitochondrial circFBXO25 were determined by qPCR assay in heart tissue and cardiomyocyte. The siRNA and adenovirus of circFBXO25 were used to respectively down-regulate or up-regulate the expression of circFBXO25. Besides, the cardiac cell death was detected by PI staining and LDH release, and the mitochondrial function was

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demonstrated by JC-1 staining, TMRM staining, Calcein-AM staining, and ATP content assay. In addition, the siRNA of TOM or PNPase were operated to detect the roles of TOM and PNPase in transporting circFBXO25 into mitochondria. Moreover, the Pulldown, mass assay and RIP assay were used to screen out and verified the ATP5PO as the targeting protein of circFBXO25. And the co-immunoprecipitation was operated to determine the interaction between ATP5PO and CypD.

Results: In this study, more than 3000 circRNAs located in mitochondria are found to be rich in the mitochondria of heart tissue. In addition, our results show that mitochondrial circFBXO25 encoded by nuclear genes is involved in the regulation of mitochondrial dysfunction and cardiomyocyte necrosis induced by hydrogen peroxide and ischemia/reperfusion respectively in vitro and in vivo. Moreover, we confirmed that circFBXO25 deficiency would further promote cardiac necrosis and mitochondrial dysfunction. Besides, the data also demonstrates that circFBXO25 overexpression protects cardiac mitochondria from oxidative stress by inhibiting the interaction between ATP5PO and CypD in vitro and in vivo. Additionally, our data demonstrate that Tom and PNPase are the essential regulators of entry of circFBXO25 into mitochondria.

Conclusion: The mitochondrial located circFBXO25 which is encoded by nuclear genome is a key positive regulator of protecting from cardiac oxidative stress injury, and TOM and PNPase regulate the process of circFBXO25 transporting into mitochondria. And circFBXO25 attenuates the cardiac injury through inhibiting the interaction between ATP5PO and CypD.

Keywords: mitochondrial circRNA, cardiovascular disease, ischemia/reperfusion injury, ATP5PO, transportation

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The mechanism of intermittent hypoxia regulating ion homeostasis against ischemia reperfusion injury in cardiomyocytes

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Ischemic heart diseases are the most common causes of debilitating disease and death worldwide. The current treatment strategy is to restore the blood supply of the ischemic myocardium as early as possible. However, the restoration of the coronary vessel flow to the ischemic myocardium within a certain period would cause more serious damage to the original ischemic myocardium; known as myocardial ischemia/reperfusion injury (MIRI). The pathological mechanism of MIRI is associated with oxidative stress, intracellular calcium overload, ferroptosis, etc. which eventually cause pathological ventricular remodeling. Previous studies have suggested that short-term intermittent hypoxia (IH), a state of repeated hypoxia–reoxygenation alternating with periods of normoxia, can serve as a conditional method to apply before or after the I/R injury to prevent MIRI. It has been demonstrated that IH can upregulate the expression of several antioxidant genes, preserve intracellular proton (pHi), induce the production of Nitric Oxide (NO), and inhibit Na^+/H^+ exchanger (NHE) via several signal transduction pathways. The aim of this study was to determine that IH-protected MIRI through enhancing the antioxidant capacity via inhibition of NHE through the NO/cyclic GMP (cGMP)/phosphokinase G (PKG)-mediated inhibition of NHE. Adult male Sprague Dawley rats weighed between 250-400g were put inside a cylinder chamber and treated with an oscillating O_2 concentration with 20% for 45 sec and 5% for 35 sec for IH. The rat heart was quickly excised, mounted on the Langendorff perfusion apparatus, and perfused with Krebs–Henseleit buffer, which was pre-oxygenated with oxygen/carbon dioxide mix gas and bathed at 37°C. The heart underwent 30 min of stability, 45 min of ischemia, and 1 h of reperfusion to induce IR injury. Primary rat neonatal and adult cardiomyocytes were cultured in IH condition with an oscillating O_2 concentration between 20% and 5% every 30 min. MTT assay was conducted to examine the cell viability. Annexin V-FITC and PI were stained to detect the occurrence of apoptosis. The NO, cGMP, and PKG Elisa kit were used to determine the intracellular NO, cGMP, and PKG level. BECEFand real-time flow cytometry were used for intracellular pH change measurement. No synthase (NOS) inhibitors, LNAME and LNMMA were used for inhibition of NO, and guanylyl cyclase (GC) inhibitors, LY83583 and KT-5823, were used for inhibition of cGMP synthesis and PKG kinase activity, respectively. Our result showed that IH significantly attenuated the cell death and heart infarction ratio caused by IR, and these effects were abolished by inhibition of NOS, GC, and PKG. Furthermore, IH could reduce the H^+ excretion rate via inhibiting the NHE activity through increasing the Na^+ concentration mediated by up-regulating the NCX activity via increasing the intracellular NO level. In conclusion, our findings suggest that IH protected the cardiomyocytes against the I/R-induced cell death via inhibition of NHE mediated by the NO/cGMP/PKG pathway.

Keywords: intermittent hypoxia, ischemic reperfusion injury, heart, NO synthesis

P3-26

Tyrosine residue phosphorylation in mitochondrial creatine kinase (CKMT2) protect from cardiac ischemic injury

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Ischemic cardiomyopathy (ICM)-related heart failure is one cause of cardiovascular disease-related deaths. Ischemic preconditioning (IPC) was found to effectively rescue ICM. IPC occurs when short-time ischemia is applied before ischemia/reperfusion injury (I/R). This brief time applied may contribute to rapid changes in protein expression and regulation emphasizing protein function modulation by post-translational modifications is important. Mitochondria play a key role in heart disease progression and can be promising target for ICM treatment. We focused on mitochondrial creatine kinase (CKMT2) under I/R injury. Sprague-Dawley rat hearts were used in an *ex vivo* Langendorff system to simulate normal perfusion, I/R, and IPC conditions. Samples were subjected to phosphoproteomic analysis. Human cardiomyocyte AC16 cells were used to investigate CKMT2's role through overexpression and how site-directed mutagenesis of CKMT2 phosphorylation sites affects cardioprotection by measuring protein activity, mitochondrial function and protein expression. CKMT2 dephosphorylates during ischemia and I/R but remained phosphorylated under IPC conditions. CKMT2 overexpression increased cell viability and mitochondrial ATP level against hypoxia/reoxygenation confirming the cardioprotective potential of CKMT2. There was decreased cell viability and increased ROS production during I/R in phosphomutated CKMT2. CKMT2 overexpression increased mitochondrial function via the proliferator-activated receptor γ coactivator-1α/estrogen-related receptor-α pathway. CKMT2 overexpression regulated mitochondrial function which led to cardiac cell protection during H/R in an in vitro setting. Mutated CKMT2 phosphorylation sites significantly decreased CKMT2 activity and mitochondrial

function especially during H/R. Our study offers a different perspective on cardioprotection during I/R injury through a novel target rather than the conventional method of targeting infarct size reduction.

Keywords: ischemia, cardioprotection, mitochondrial creatine kinase, mitochondria, phosphorylation

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Marine compound neopetroside a protects the heart from ischemia/reperfusion injury through gsk3ß inhibition

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Background: Recent trends suggest novel natural compounds as promising treatments for cardiovascular disease. We investigated the role of neopetroside A (NPS A), a natural marine pyridine nucleoside, on mitochondrial metabolism and heart function and determined its cardioprotective role against ischemia/reperfusion (I/R) injury.
Methods and Results: NPS A reduced *ex vivo* I/R-induced damage in rat hearts by preserving hemodynamics and mitochondrial respiration capacity. NPS A significantly suppressed cardiac fibrosis in mice after left coronary artery ligation myocardial infarction surgery *in vivo*. *In vitro* analysis showed high cellular and mitochondrial function in NPS A–treated rat H9c2 cells treated, with increased glycolysis, oxidative phosphorylation, NAD+/NADH ratio, and metabolic processes compared to untreated cells. *In vitro* kinase activity assays showed that NPS A inhibited GSK-3β. Docking simulation studies and surface plasmon resonance binding assays revealed interactions of NPS A with GSK-3β. NPS A increased the NAD+/NADH ratio via the NRF2/NQO1 pathway, revealing a mechanism underlying the effects of NPS A on metabolic and cellular processes.
Conclusion: NPS A enhances mitochondrial metabolism and may serve as a potential treatment against acute I/R damage and myocardial fibrosis inhibiting GSK-3β.

Keywords: GSK3ß, NPS A, I/R injury, mitochondria, cardioprotection

P3-28

Zinc overload induces damage to H9c2 cardiomyocyte through mitochondrial dysfunction and ros-mediated mitophagy

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Zinc is a trace metal element essential for maintaining redox balance, cell proliferation, and apoptosis. However, excessive zinc exposure can be toxic, leading to mitochondrial dysfunction. In this study, we established a zinc overload model by treating rat cardiomyocyte H9c2 cells with $ZnCl_2$ at different concentrations. Our results showed that zinc overload caused cellular damage, including reduced cell survival rate, increased LDH and reactive oxygen species (ROS) levels, decreased mitochondrial membrane potential and impaired mitochondrial function and dynamics. Furthermore, zinc overload induced mitochondrial autophagy by activating the PINK1/Parkin signaling pathway via ROS, while NAC inhibited mitophagy and weakened the activation of PINK1/Parkin pathway by zinc overload, thereby preserving mitochondrial biogenesis. In addition, our data also showed that Mfn2 deletion induced increased ROS production and exacerbated cytotoxicity induced by zinc overload. Our results therefore suggest that $ZnCl_2$-induced ROS generation causes mitochondrial autophagy and mitochondrial dysfunction, damaging H9c2 cardiomyocytes. Additionally, Mfn2 may play a key role in zinc ion-mediated endoplasmic reticulum and mitochondrial interactions.

Keywords: zinc overload, reactive oxygen, mitophagy, mitofusin-2, H9c2

P3-29

Mechanism of circpum1 in regulating mitol in ferroptosis-mediated myocardial injury

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Background: Ischemia-reperfusion (I/R) is a severe cardiac condition associated with high morbidity and mortality rates. Recent studies have highlighted ferroptosis, a regulated form of cell death characterized by iron-dependent lipid peroxidation, is a significant contributor to cardiovascular diseases. Circular RNAs (circRNAs) have emerged as crucial regulators in various biological processes, including cardiovascular pathologies. However, the involvement of circRNAs in the regulation of ferroptosis-mediated myocardial injury remains poorly understood.
Methods: Second generation sequencing was used for circRNA screening; Pull-down and RIP was used to verify the combination of circPUM1 and MITOL(Mitochondrial E3 Ubiquitin Protein Ligase); Immunoprecipitation (IP) was used to identify MITOL as an interacting protein of FLNA. Lipidomic analysis was used to determine how iron affected the amount of polyunsaturated fatty acids; In addition, we overexpressed and knocked down circPUM1, MITOL and FLNA to verify their biological functions. Hypoxia-reoxygenation (H/R) model and ischemia-reperfusion injury (I/R) model were used to determine whether iron was involved in this process.
Results: Our findings demonstrate that circPUM1 is significantly upregulated in cardiomyocytes hypoxia-reoxygenation model, and its regulatory pathway is associated with ferroptosis. Knockdown of circPUM1 resulted in a reduction in cell death caused by ferroptosis in cardiomyocytes, as evidenced by decreased lipid peroxidation and preserved mitochondrial integrity. Mechanistically, we discovered that circPUM1 binding to MITOL, relieving its inhibitory effect on FLNA. This, in turn, leads to increased FINA expression, promoting mitochondrial dysfunction and intensifying ferroptosis in myocardial cells. Furthermore, FLNA will affect the nuclear translocation of STAT3 then it influences ferroptosis at the transcriptional level.
Conclusion: This study provides novel insights into the regulatory mechanism of circPUM1 in ferroptosis-mediated myocardial injury. Our results suggest that circPUM1 affects the expression of FLNA by binding MITOL protein, leading to altered nuclear entry of STAT3, and influences cardiomyocytes ferroptosis. Understanding these molecular mechanisms may pave the way for the development of targeted therapeutic interventions to mitigate the detrimental effects of I/R.

Keywords: circPUM1, MITOL, ischemia-reperfusion, ferroptosis, FLNA

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Fisetin and kaempferol protect heart from myocardial ischemia-reperfusion injury through the regulation of ferroptosis

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Myocardial ischemia-reperfusion injury (MIRI) is a lethal disease. Finding new solutions to treat I/R injury has long been an important issue. Ferroptosis, characterized by iron-dependent accumulation of lipid peroxide, has been found to participate in I/R injury. Flavonols, such as Fisetin (FIS) and Kaempferol (KMP), are natural compounds found in many edible plants showing lipophilic antioxidation and iron chelation activity. FIS and KMP have been shown to exert anti-apoptosis, mitochondrial protection, anti-inflammation, and oxidative stress reduction in cardiomyocytes. However, the effects of FIS and KMP on ferroptosis and MIRI are still unclear. Thus, we aimed to investigate whether FIS and KMP exerted anti-ferroptotic effects in cardiomyocytes and could prevent MIRI. Our results show that FIS and KMP could ameliorate cell death induced by Erastin (Era), RSL3, and Iron complex (FeSP) through reducing the levels of intracellular ROS, lipid peroxide, and ptgs2 mRNA in H9c2 and neonatal cardiomyocytes. FeSP treatment increased the intracellular ferrous iron level, and this effect was suppressed by KMP, but not FIS. Intriguingly, treatment duration exhibits different effects on ferroptosis-related protein levels. Treatment with FeSP for 2 h, the levels of GPX4 and FTH protein were reduced, and these effects were abolished by co-treatment with FIS or KMP. The FeSP-increased ACSL4 was not affected by FIS and KMP. Treatment with FeSP for 24 h, however, the levels of GPX4 and FTH protein were increased, and the FeSP-increased GPX4, but not FTH, was abolished by co-treatment with FIS or KMP. We also investigated the protein levels and nuclear translocation of Nrf2 and HO1. Treatment with FeSP for 2 or 24 h, the Nrf2 protein levels were increased, and these effects were abolished by co-treatment with FIS or KMP. FeSP time-dependently increased the HO1 protein levels, and these effects were abolished by co-treatment with FIS or KMP. Nrf2 nuclear translocation was found in 24 h but not 2 h FeSP treatment suggesting increased transcription of downstream proteins, HO1, FTH and GPX4 protein levels. The MIRI model was done with Langendorff isolated heart perfusion system. FIS and KMP significantly reduced the infarct size and the levels of FTH, NCOA4 and GPX4 protein and increased the heart contractility as compared to the MIRI group. In conclusion, we proved that FIS and KMP are effective ferroptosis inhibitors in cardiomyocytes and could prevent MIRI through inhibiting ferroptosis and infarct size and improving cardiac functions.

Keywords: myocardial ischemia-reperfusion injury, ferroptosis, flavonols, fisetin, kaempferol

P3-31

Arachidonic acid induces lipid peroxidation and lysosomal membrane permeabilization in H9c2 myoblasts

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Background: Previous studies showed that the accumulation of arachidonic acids (AA) in the ischemic-reperfused cardiac tissue can trigger intracellular iron disturbance, apoptosis, and ferroptosis in cardiomyocytes. Ferroptosis is programmed cell death caused by iron accumulation and enhanced lipid peroxidation, which in turn resulted in increased lysosomal membrane permeability (LMP). Autophagy, a lysosome-dependent degradation pathway, is an important clearance pathway to remove damaged organelles and misfolded or aggregated proteins. To date, whether AA-induced lipid peroxidation is the key factor for the occurrence of LMP in cardiomyocytes remains unclear. This study aimed to identify the specific events occurred during the AA-induced LMP process, including the production of reactive oxygen species (ROS), and the relationship between lipid peroxidation and LMP.
Methods: H9c2 myoblasts were used for this study. Cells were loaded with Acridine Orange, and lysosomes were measured using multi-mode microplate reader and confocal microscopy. Intracellular iron and ROS were detected by FerroOrange/multi-mode microplate reader and DCF-DA/confocal microscopy, respectively. Lipid peroxidation was detected using C11-BODIPY and flow cytometry.
Results: Our results showed that treatment with AA (50 mM) for 30 min increased intracellular iron accumulation. Moreover, treatment with AA (50 mM) for 60 min induced an increase of ROS, and this effect was inhibited by dithiothreitol, MnTMPyP, and N-(2-mercaptopropionyl)-glycine, but not Ferrostatin-1 (Fer-1) and Liproxstatin-1 (Lip-1). Lipid peroxidation and lysosomes permeabilization were also observed after treatment with AA (50 mM) for 60 min, and this effect was blocked by Fer-1 and Lip-1.
Conclusion: Our results suggest that lipid peroxidation played an important role in the AA-induced LMP in cardiomyocytes, and this effect might be due to the accumulation of iron and ROS.

Keywords: arachidonic acid, lysosome, ROS, lipid peroxidation

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Effect of cyanocobalamin supplementation on connexin-43 protein levels in the rat cardiac ventricles

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Background: Cyanocobalamin (Vitamin B12) *Cyanocobalamin* has been investigated have an effect to increase heart cell differentiation through expression of connexin which affect to conduction system. In the literature, cyanocobalamin was involved in promoting the conduction system between cells through the expression levels of a gap junction protein called connexin. Connexin-43 (Cx43) was the main gap-junction protein in the atrial and ventricular myocardium of the heart. Previous studies showed that when Vitamin B12 (0.5 mM) was added to the medium on day 3 of culture, Cx40 expression levels increased compared to controls. In the heart, gap-junctions contribute to the cell-to-cell action potential conduction system. In some cardiac diseases, connexin expression was altered and was associated with an increased tendency to arrhythmias. This research aims to Analyzing the role of oral and intramuscular vitamin B12 supplementation on levels of Connexin-43 in the rat cardiac ventricles tissue.
Methods: This research was an experimental design using 39 male wistar rats aged 12 weeks. Rats were divided into 3 groups each n = 13, control group, group B12 orally and group B12 intramuscular injection. Rats were given vitamin B12 everyday for 6 weeks. Assessment of the cardiac conduction system was carried out by performing connexin-43 ELISA examinations on rat heart ventricles.

Results: After treatment with vitamin B12 supplementation for 6 weeks, the average vitamin B12 level in the control group was 317.7 pg/dl, the oral B12 group was 336.1 pg/dl and the B12 I.M group was 352.3 pg/dl. The mean Connexin 43 (Cx-43) level in the control group was 8.34 ng/ml, the oral B12 group was 9.33 ng/ml and the B12 I.M group was 9.31 ng/ml. T-dependent test found that there was a significant difference in plasma Vit B12 levels between before and after oral Vit B12 administration (p-value 0.000). The results of the Will Thomson/Coxon test show that there is a significant difference in plasma Vit B12 levels between before and after administration of Vit B12 I.M (p-value 0.001). The results of the T-dependent test showed that there was a significant difference in Cx-43 levels between the B12 I.M group and the control group (p-value 0.004), while there was no significant relationship between the Cx-43 levels between the control group and the oral B12 group (p=0.127).
Conclusion: This study showed that vitamin B12 supplementation for 6 weeks increased rat cardiac ventricular Cx43 levels.

Keywords: cardiac conduction, connexin-43, Cyanocobalamin, ventricle, vitamin B12

P3-33

Cardiac efficiency and starling's law of the heart

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Background: The formulation by Ernest Starling in 1914 of The Law of the Heart states that "*the [mechanical] energy of contraction, however measured, is a function of the length of the muscle fibre*". Starling later, in 1927, also stated that "*the oxygen consumption of the isolated heart … is determined by its diastolic volume, and therefore by the initial length of its muscular fibres*". The field has since been left unclear as to whether cardiac efficiency is a function of muscle length. This study was motivated to extend Starling's Law of the Heart to include consideration of the efficiency of contraction. Recent improved understanding gained in the field of the factors, including the distinct effects of preload and afterload, that affect cardiac efficiency presents an opportunity for us to investigate the elusive length–dependence of cardiac efficiency.
Methods: In this study, we assessed both mechanical efficiency and crossbridge efficiency by measuring the heat output of isolated rat ventricular trabeculae performing force–length work–loops over ranges of preload and afterload. The combination of preload and afterload allowed us, using our modelling frameworks for the end–systolic zone and the heat–force zone, to simulate cases by recreating physiologically feasible loading conditions.
Results: We found that across all cases examined, both work output and change of enthalpy increased with initial muscle length; hence it can only be that the former increases more than the latter to yield increased mechanical efficiency. In contrast, crossbridge efficiency increased with initial muscle length in cases where the extent of muscle shortening varied greatly with preload.
Conclusion: We conclude that the efficiency of cardiac contraction increases with increasing initial muscle length and preload. An implication of our conclusion is that the length–dependent activation mechanism underlying the cellular basis of Starling's Law of the Heart is an energetically favourable process that increases the efficiency of cardiac contraction.

Keywords: Frank-Starling mechanism, cardiac force-length relation, cardiac energetics, mechanical efficiency

P3-34

Cellular ultrastructural remodelling compensates for bioenergetic dysfunction to maintain force production in diabetic heart tissues

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Diabetic cardiomyopathy is associated with dysfunctions in metabolic and mechanical performance. The aetiology of the disease remains unclear, particularly, in type 2 diabetes (T2D) where compensatory mechanisms may obscure underlying disease pathways. In this study, we constructed 2D computational models of cardiomyocyte cellular ultrastructure to investigate the effects of T2D diabetes on the coupling between cardiac bioenergetics and contractile function. T2D rats were induced with a high-fat diet and a single low-dose injection of streptozotocin. Control animals had a standard chow diet and received a sham injection of citrate buffer. Blocks of wall tissues (n = 18 for each group) and free-running trabeculae (n = 14 for each group) were dissected from the left ventricle for transmission electron microscopy (TEM) imaging and *in vitro* mechanical experiments, respectively. Using the TEM images, cell-specific 2D finite element models of cardiomyocyte ultrastructure were generated by semi-automatic segmentation of myofibrillar and mitochondrial regions. Models of cross-bridge cycling dynamics and mitochondrial respiration were integrated in nodes located within the myofibrillar and mitochondrial areas, respectively. Simulations were performed to assess the effects of T2D-induced changes in mitochondrial structure and function on the steady-state distribution of metabolites, and the subsequent effects on force production. The simulated twitch force response was validated against active force production from trabeculae contracting at 3 Hz at 37°C. In addition, a set of cross-over simulations was performed to ascertain whether changes in bioenergetics were driven by mitochondrial functional or structural changes. From the TEM images, we found that T2D myocytes had a lower mitochondrial area fraction but a higher myofibrillar area fraction compared to the controls. Our model simulations of the steady-state distribution of metabolites showed no difference for the concentrations of ATP, ADP and PCr, but an increase in Pi for the T2D group compared to control. The force production per cross-bridge was lower in the T2D group because of the increase in Pi, but the force production across the cell, which accounts for differences in myofibrillar area, was unchanged. These model predictions on contractile function were validated by our *in vitro* trabeculae experiments, which also showed no change in active stress production. Our cross-over simulations revealed that a reduction in mitochondrial function, as opposed to structure, is the primary driver of metabolic and cross-bridge dysfunction in T2D. In T2D, a reduction in mitochondrial ATP synthesis leads to an increase in cellular Pi which reduces the force production of individual cross-bridges. However, there appears to be a compensatory response whereby an increase in cellular myofibrillar area fraction overcomes the cross-bridge dysfunction such that force production at the cellular level is maintained.

Keywords: type 2 diabetes, cardiac mechanics, mitochondria, computational modeling

Poster

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P3-35

The effect of HK660S on mitochondrial function in diabetic cardiomyopathy

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Background: This study aimed to investigate the effects of HK660S drug (β-lapachone): Ameliorates cardiac dysfunction function in the diabetic mouse model by improving mitochondrial function and biogenesis and increases antioxidant activity in various tissues on diabetic mouse modal.
Methods: C57BL/6 mice were fed with the High-fat diet (HF) for 10 weeks to induce type 2 DM. Mice were fed HK660S (20mg/kg and 80mg/kg/day), positive control group treated with Metformin 200mg/kg/day with the HF treatment to investigate the protective effects of HK660S against diabetic cardiomyopathy (DCM).
Results: Lap decreased heart weight, food and water intake, increased heart function, reduced oxidative stress, improve mitochondrial content, biogenesisis, inflammation and apoptosis in diabetic mouse modal.
Conclusion: This study showed that HK660S drug can protect the heart from DCM by improving antioxidant ability, inflammation, apoptostis and mitochondrial activity in cardiomyocytes. HK660S drugs provides a potential therapeutic option for DCM.

Keywords: diabetes, cardiomyopathy, β-lapachone, high fat diet

P3-36

Effects of human TNNT2 overexpression on dilated cardiomyopathy (DCM) pathogenesis in DCM model mice with TNNT2 ΔK210 mutation

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Background: Dilated cardiomyopathy (DCM) is a myocardial disease characterized by diffuse contractile dysfunction and dilation of the ventricles. Although many gene mutations including sarcomeric proteins and/or cytoskeletal proteins have been reported as causes of DCM, we focused on the mutation of the gene encoding myocardium troponin T (TNNT2), which is the deletion of lysin at position 210 (ΔK210). It has been reported that overexpression of mutant TNNT2 in normal mouse myocardium causes cardiomyopathy by replacing normal troponin T. Therefore, we aimed to improve DCM with TNNT2 mutation by overexpressing normal TNNT2 to replace mutant troponin T with a normal one.
Methods: Experiments were performed using wild-type (WT) mice, TNNT2 ΔK210 mutation knocked-in mice (DCM model mice: DCM), and normal human cardiac troponin T overexpressed DCM mice (Tg/DCM mice). First, we investigate the replacement rate of normal human cardiac troponin T in Tg/DCM mice (n=4). Then, lifespans (n=68~95), tissue weights, expression levels of heart failure biomarker (ANP and BNP) and fibrosis biomarker (TGF-β) mRNAs, and cardiac functions by echocardiography (7 weeks, n=6~10) were analyzed to reveal the effect of normal human cardiac troponin T overexpression. In addition, a telemetry electrocardiogram measurement device was implanted in each mouse, and the number of arrhythmias in 24-hour ECG data was counted (10 weeks, n=3~5). Also, the expression levels of channel genes associated with membrane potential were examined.
Result: Approximately 40% of mutant cardiac troponin T was replaced by normal human cardiac troponin T. The lifespan of Tg/DCM mice was about 2-fold longer than that of DCM mice. Tissue weight and expression level of ANP, BNP, and TGF-β mRNAs were improved in Tg/DCM mice compared to DCM mice, but there was no significant difference in cardiac functions and ventricular wall thickness between DCM and Tg/DCM mice. The telemetry ECG analysis showed that the frequency of PVC and VT significantly decreased in Tg/DCM mice compared to DCM mice. In addition, although the gene expressions of membrane potential channels were altered in DCM mice compared to WT mice, normalized in Tg/DCM mice.
Conclusion: These results suggest that the replacement of mutant troponin T with normal troponin T ameliorates the pathogenesis of troponin T mutant DCM by suppressing ventricular arrhythmias.

Keywords: dilated cardiomyopathy, gene therapy, troponin T, arrhythmia, heart failure

P3-37

Pitx2c-conditional knockout mice may become a useful model for understanding the pathogenesis of post-capillary pulmonary hypertension

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Background: Pulmonary hypertension (PH) is an intractable disease that causes cardiopulmonary dysfunction due to elevated pulmonary arterial pressure. The pathogenesis of PH varies greatly depending on whether the primary lesion is upstream or downstream of the pulmonary capillaries, and the latter, post-capillary PH (pc-PH) caused by pulmonary vein stenosis/obstruction or left heart disease, currently has not an appropriate animal models to study its pathophysiology and pharmacological effects.
Hypothesis: We hypothesized that a tissue-specific deletion of a gene involved in the formation of pulmonary veins could create a pathological mouse model of pc-PH.
Methods: We focused on the homeobox transcription factor Pitx2c, which has been reported to be essential for pulmonary vein formation, and crossed a Pitx2c-floxed mouse line with a Cre mouse line of sarcolipin, an endoplasmic reticulum membrane protein, to obtain Pitx2c-conditional knockout (cKO) mice specific for pulmonary veins and atrial myocardium. We then examined the cardiac morphology and hemodynamics of Pitx2c-cKO mice.
Results: Tissue weights of 8-week-old Pitx2c-cKO mice showed that the relative weights of both atria (especially the right atrium), right ventricle and lungs were significantly increased in Pitx2c-cKO mice, while the relative weight of the left ventricle was decreased. Furthermore, echocardiography performed on 3-month-old Pitx2c-cKO mice revealed that the right ventricle was markedly enlarged in Pitx2c-cKO mice, with enlarged pulmonary artery annulus diameter and increased velocity time integral (VTI) of blood flow through the right ventricular outflow tract. Pitx2c-cKO mice also had significantly decreased left ventricular end-diastolic diameter (LVDd), end-systolic diameter (LVDs), and mass (LVM). On the other hand, there was no difference in left ventricular fractional shortening (%FS) or tricuspid annular plane systolic excursion (TAPSE), indicating no cardiac dysfunction in Pitx2c-cKO mice. Cardiac catheterization also revealed elevated right ventricular pressure in some Pitx2c-cKO mice.
Conclusion: These primary findings suggest that the Pitx2c-cKO mice

may be a model for understanding the pathogenesis of pc-PH.

Keywords: pulmonary hypertension, Pitx2c, conditional knockout mice, pathological model

P3-38

Cereblon as potential biomarker for diabetic cardiomyopathy in type 2 diabetes-induced mice

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The high incidence of heart failure in patients with type 2 diabetes mellitus (T2DM) can be attributed to myocardial fibrosis, weakening of ventricular function, and damage to myocardial muscles, such as mitochondrial dysfunction. We studied physiological changes in the heart, which are early characteristics of diabetic myocardial disease, using 6- and 8-week old obese T2DM model mice (db/db, BKS.Cg-*Dock7*^{m}+/+*Lepr*^{db}/J) and wild type mice. We confirmed mitochondrial dysfunction in 8 weeks old db/db mice, which was not accompanied by any changes in heart function. Metabolomic analysis performed on heart tissues revealed that the levels of 12 metabolites changed substantially. The levels of glucose and leucine increased considerably, and lipid metabolites changed. Protein expression analysis of the blood and heart tissues confirmed a remarkable increase in cereblon (CRBN) levels and a decrease in AMP-activated protein kinase, a negative regulator. Furthermore, in silico studies on data in the blood of diabetic cardiomyopathy (DCM) patients. These results show that an increase in CRBN, without any abnormal heart function, in early T2DM mice roles an important role in the reduction of mitochondrial function and metabolomic changes. Therefore, CRBN level in blood and heart tissues may serve as a diagnostic biomarker for the detection of early DCM.

Keywords: Cereblon, diabetic cardiomyopathy, type 2 diabetes, AMPK, CRBN

P3-39

Innovative application of artificial intelligence-based skeleton model to detect cardiopulmonary fitness in seniors

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The American Heart Association considers cardiopulmonary fitness (CRF) an independent predictor of health and survival outcomes. The 2-Minute Step Test (2-MST) now is used in Taiwan as a measurement of cardiopulmonary fitness in seniors. However, continuous measurements may cause human errors. Since artificial intelligence (AI) has been utilized to establish a skeletal model through deep learning, the model coupled with algorithm analysis (such as OpenPose and MediaPipe-pose) becomes a common tool to detect human body movements. In comparison, OpenPose is a real-time multi-person system, and MediaPipe is a real-time one-person system. Up to date, it has not been used to conduct repeated measurements for assessment purposes. Thus, the study was designed to explore if the skeletal model coupled with algorithm analysis may be used to conduct a 2-minute step test for assessing CRF in seniors. Accordingly, Aim1 was to explore the feasibility of the skeleton model coupled with OpenPose to conduct the 2-minute test in the young. Aim2 was to apply the same system to evaluate the accuracy of AI-based detection in seniors. Aim3 was to modify the environment to further improve the accuracy. Aim 4 was to compare the detection accuracy between OpenPose and MediaPipe systems in seniors. We define accuracy as the AI counts divided by manual counts. The overall correct rate was defined by the number of subjects with an accuracy between 0.9 and 1.1 divided by the total number of subjects. Our results show that the accuracy of OpenPose-based detection was 0.84±0.3 in the young and the correct rate was 0.68. The accuracy of OpenPose-based detection for the 2-MST was 0.39±0.39 and the correct rate was 0.11 for seniors. After modifying the environment, the accuracy of OpenPose-based detection for the 2-MST increased to 0.84±0.3 and the correct rate was 0.43 in senior. The MediaPipe-based detection coupled with AR goggles significantly increased the accuracy from 0.84±0.3 to 0.95±0.17; the correct rate increased from 0.43 to 0.9. As our results suggested, the AI-based skeleton model coupled with the MediaPipe algorithm and AR goggles can indeed serve as an effective tool for the 2-MST and predict the CRF in seniors.

Keywords: cardiopulmonary fitness (CRF), 2-minute step test (2-MST), senior, OpenPose, MediaPipe

P4-1

Acute effects of PM2.5 on airway inflammation in type 2 diabetes mellitus patients in Northern Thailand

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Type 2 diabetes mellitus (T2DM) is a chronic non-communicable disease with multiple systemic complications. The respiratory system is one of the most systems affected by T2DM, such as decreased lung function and respiratory muscle strength and an increase in airway inflammation. Northern of Thailand has seen an increase in air pollution every year, especially particulate matter (PM) 2.5. The increase in PM2.5 leads to increased exacerbation and hospitalization in patients with chronic non-communicable diseases. However, the effects of acute PM2.5 exposure on airway inflammation of T2DM patients, as measured by fractional exhaled nitric oxide (FeNO) levels, have not been investigated. Therefore, the aims of this study were to determine the impact of acute PM2.5 exposure on airway inflammation and association between PM2.5 and airway inflammation in patients with T2DM.This study was carried out on 40 diagnosed T2DM patients aged 20 to 60 years. They were divided into two groups: the controlled T2DM group (n=20) and the uncontrolled T2DM group (n=20). All participants underwent FeNO testing during acute PM2.5 exposure. FeNO level was higher in patients with uncontrolled T2DM compared to controlled T2DM patients (12.09 ± 3.94 vs 37.51 ± 11.28, $P < 0.001$). Linear regression analyses revealed a positive correlation between FeNO and PM2.5 levels ($r = 0.430$, $P < 0.01$) in uncontrolled T2DM patients. In the controlled T2DM patients, there was no significant difference between FeNO and PM2.5 levels. These findings indicate that acute PM2.5 exposure increased airway inflammation in uncontrolled T2DM patients.

Keywords: type 2 diabetes mellitus, PM2.5, airway inflammation

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P4-2

Effect of air pollution on cardiorespiratory fitness in patients with hypertension in Northern Thailand

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Peak oxygen consumption (VO_2peak) and six-minute walk test (6MWT) are important indices of cardiorespiratory fitness. VO_2peak and 6MWT were lowered in patients with hypertension. Recently, air pollution produced by particulate matter (PM) both PM2.5 and PM10) is a serious health issue that occurs in Northern Thailand. However, the correlation of air pollution to cardiorespiratory fitness in hypertensive patients has not been intensively investigated. The present study measured the influence of air pollution on VO_2peak and 6MWT in 30 hypertensive patients (systolic blood pressure 148.17 ± 11.28 mm Hg, diastolic blood pressure 94.30 ± 12.75 mm Hg) with ages ranging from 24 to 74 years old (22 males, 8 females). Each subject completed 6MWT, and the total distance of 6MWT was used to calculate VO_2peak. PM2.5 and PM10 concentrations ranged from 43.10 to 143.80 $\mu g/m^3$ and 100.20 to 399.50 $\mu g/m^3$, respectively. Mean VO_2peak and 6MWT showed 30.69 ± 4.72 ml/min/kg and 463.49 ± 130.57 m, respectively. Linear regression analyses revealed a negative correlation between VO_2peak and PM2.5 ($r = -0.380$, $p<0.05$) and PM10 ($r = -0.452$, $p<0.05$). Moreover, 6MWT in hypertensive patients was inversely related to air pollution as assessed by PM2.5 ($r = -0.637$, $p<0.001$) and PM10 (-0.691, $p<0.001$). The results suggest that cardiorespiratory fitness in hypertensive patients decreases during higher concentrations of air pollution.

Keywords: hypertension, PM2.5, PM10, cardiorespiratory fitness

P4-3

PGE_2 and PGD_2 signaling pathways mediate the antifibrotic effects of Gas6 in bleomycin-induced lung fibrosis

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Emerging evidence suggests that the EMT process is a major event in idiopathic pulmonary fibrosis (IPF) pathogenesis. We previously demonstrated that growth arrest-specific protein 6 (Gas6) contributes to alveolar epithelial cell homeostasis by regulating proliferation, tissue repair, and the epithelial–mesenchymal transition (EMT) in vitro. Here, we investigated whether COX-2-derived PGE_2 and PGD_2 signaling pathways play a role for mediating anti-EMT effects of Gas6 in bleomycin (BLM)-induced lung fibrosis. Gas6 was given intraperitoneally one day before BLM treatment and then administrated once in 2 days. The COX-2 inhibitor NS-398, PGE_2- or PGD_2-specific receptor antagonists, such as antagonists of E-prostanoid-2 receptor (EP2) (AH-6809), or DP2 (BAY-u3405) were coadministered with Gas6 one day before BLM treatment and then administrated once/day (AH6809) or once in 2 days (NS398 and BAY-u3405) for 14 days after BLM treatment. Coadministration of NS398, AH6809, or BAY-u3405 significantly reversed anti-EMT effects of Gas6 administration, including inhibition of E-cadherin loss, reduction of synthesis of N-cadherin and α-SMA at the mRNA levels, and restores of the mRNA abundance of Snai1, Zeb1, and Twist1 in primary alveolar type II epithelial cells (ATII). We also found that coadministration of these antagonists reversed the decreases in mRNA expression levels of fibroblast activation markers, including α-SMA, collagen type 1, and fibronectin, in the primary fibroblasts by administration of Gas6 at 14 days post-BLM treatment. Taken together, these data suggested that enhanced COX-2-derived PGE_2 and PGD_2 signaling pathways contribute to the inhibition of EMT process and fibroblast activation by administration of Gas6 in BLM-induced fibrosis.

Keywords: Gas6, lung fibrosis, PGE2, PGD2, EMT

P4-4

Cerebral neural oscillations associated with sensory gating of respiratory mechanosensation

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Human respiratory sensory gating is a neural process associated with inhibiting repetitive respiratory mechanical stimuli in the central nervous system. With a paired transient inspiratory occlusion paradigm, respiratory sensory gating is demonstrated by a diminished N1 peak amplitude to the second stimulus (S2) relative to the first stimulus (S1) in scalp-recorded respiratory-related evoked potential (RREP). However, the role of brain neural oscillations in respiratory sensory gating remain unclear. The purpose of the present study was to investigate mechanical stimulus elicited respiratory sensory gating with time-frequency analyses.

A group of healthy human subjects were recruited to participate in the study. Two transient 150-ms inspiratory occlusions with a 500-ms interstimulus-interval were administered every 2 to 4 breaths. The subjects were instructed to count the number of respirations occluded during the experiment. At least 100 paired inspiratory occlusions were collected for data analysis. The perceived level of breathlessness and level of unpleasantness of the occlusions were measured after the experiment. The RREP N1 peak amplitudes were identified, and neural oscillations were extracted using a complex Morlet wavelet in the BrainVision Analysis software 2.0. Measures of the evoked and induced oscillations were obtained for data analysis. The differences in peak-to-peak amplitude of RREP N1 component and time-frequency power between S1 and S2 were compared via 2-tailed paired t-tests with an alpha level of 0.05. Pearson correlational analysis was used to examine the relationship between the subjective rating of perceived breathlessness intensity/unpleasantness and the neural oscillations power bands. All statistical analyses were performed using JAMOVI software version 2.2.

The results revealed that the RREP N1 peak amplitude to the S1 was significantly larger than to the S2. For both the evoked and induced oscillations, time-frequency analysis showed higher theta activations in response to S1 relative to S2. The averaged respiratory sensory gating S2/S1 ratio for the N1 peak amplitude was 0.71. A positive correlation was observed between perceived unpleasantness and induced theta power.

Our results suggested that theta oscillation reflects the "gating" phenomena in respiratory sensation regardless of evoked or induced powers. In addition, the induced theta oscillation may be a useful parameter in predicting the emotional aspect of respiratory sensation of mechanical stimulus. The findings serve as a foundation for future investigations in the underlying mechanisms of respiratory sensory gating, particularly in diseased populations.

Keywords: respiratory sensation, dyspnea, respiratory sensory gating, cerebral activation, neural oscillations

P4-5

Production of animal stealth red cells by cell surface modulation

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Background: Blood transfusion has been practiced for centuries to save human and animal lives. As the pet market continues to expand, the demand for blood transfusions in animals is steadily increasing. However, similar to the situation in humans, there is a shortage of minor blood groups in animals. In the case of cats, the AB blood group system has reported three blood groups. Blood type A is the most prevalent, while blood type B is found in only 1% of the feline population in Korea. Type B cats naturally develop antibodies against type A antigens, which can lead to potentially fatal hemolytic reactions if transfused with incompatible blood. To address this challenge, the manipulation of red blood cell surface antigens using methoxy polyethylene glycols (mPEGs) offers a promising approach to create "stealth" red cells that do not bind anti-A antibodies. This study aims to determine the optimal conditions for producing feline stealth red cells and validate their stealth effect in vitro.
Methods: Feline red cells of type A were subjected to PEGylation using mPEG, and the PEGylation reaction conditions were optimized. Subsequently, the physical properties of the cell membrane and the biochemical properties of the pegylated red cells were assessed, followed by the evaluation of phagocytosis mediated by anti-A antibodies.
Results: The feline stealth red cells were generated with mPEG of 2 mM, 20 kDa as determined by aggregation reaction. When reacted with feline A antibodies in vitro for up to 24 hours, the Stealth red cells showed minimal or no aggregation reaction. Cell membrane stability for cell viability and biochemical properties related to oxygen transport capacity of Stealth red cells were not different from normal feline A typed red cells.
Conclusion: It is the first report in veterinary medicine showing that manipulation of feline A erythrocytes with activated mPEG can produce universal erythrocytes suitable for transfusion to B-typed cats.

Keywords: stealth RBC, membrane modification, methoxy polyethylene glycol, immune camouflage

P4-6

Effect of chronic hyperglycemia and SGLT 2 inhibitors on the acute lung injury

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Background: ARDS is associated with significantly increased morbidity, mortality and usage of critical care resources. There are so many modifiers of ARDS, diabetes is one of them. Earlier researches showed that diabetes may delay or may increase the chances of ARDS. So, to know the exact association of Diabetes and ARDS is very important for the treatment of ARDS. To confirm the findings effect of antihyperglycemic agent Empagliflozin (SGLT2 inhibitors) on pulmonary parameters and survival times were used.
Objective: To study the effect of chronic Hyperglycemia and SGLT 2 inhibitor on acute lung injury.
Methods: The experiments were performed on healthy adult male albino rats weighing 150-180 gm. The trachea, jugular vein and carotid artery of anaesthetized rats were cannulated to keep the respiratory tract patent, deliver saline/ drugs and record BP, respectively. Animals were randomly divided into three groups. Acute lung injury (ALI) was induced in all three groups by injecting oleic acid and Cardio-respiratory parameters, pulmonary water content and histological examination of the lung was done
Control: Fed with high fat diet for 2 weeks
In group II: Hyperglycemic rats, fed with high-fat diet for 2 weeks followed by streptozotocin(35mg/kg i.p) injection,
Group III: Hyperglycemic rats were treated with oral dose of 30mg/kg of empagliflozin
Results: OA-induced ALI in the hyperglycemic animal model showed early deterioration of all cardiorespiratory parameters, and histological examination of the lungs showed moderate focal interstitial fibrosis, alveolar septal infiltration, alveolar oedema, alveolar exudate and peribronchial inflammatory cell infiltration. The survival time of animals in this group is less compared to the Control group. Whereas, rats treated with empagliflozin showed improved pulmonary parameters, survival time and histology shows less damage to the lung.
Discussion: The hyperglycaemia induced increased DNA damage, impaired DNA repair, increased oxidative stress, elevated inflammation by increasing proinflammatory cytokines may be attributed to increased Lung injury, early deterioration of cardiopulmonary parameter and early death of ALI rats. Hyperglycemia also induces formation of advanced glycated end products which promote inflammation and endothelial dysfunction. However, empagliflozin (SGLT2 inhibitor) may improve the condition by promoting Natriuresis and glucosuria, leading to lowering of cardiac preload thereby reducing pulmonary congestion and systemic oedema. It also acts as anti-inflammatory by increasing weight loss and reducing inflammation of adipose tissues, may improve endothelial function by inhibiting inflammatory pathways through blocking interleukin- 1beta stimulated cytokine secretion.
Conclusion: OA-induced ALI in the hyperglycemic animal model shows early deterioration of all cardiorespiratory parameters which is improved by antihyperglycemic drug empagliflozin.

Keywords: diabetes, ARDS, SGLT-2 Inhibitor, hyperglycemia, oleic acid

P4-7

Pulmonary function test of young healthy high-altitude dwellers in eastern nepal

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Background: More than a million people live at high altitude in Nepal. The primary problem at high altitude is low oxygen concentration affecting pulmonary and other functions. There is paucity of pulmonary function test (PFT) reports of highlanders in Nepal. Thus, this study was conducted to study PFT of healthy high-altitude dwellers living at around 3000 m altitude in eastern Nepal and their correlation with demographic data retrospectively.
Methods: The study included all recorded data of PFT from healthy native highlanders living at Gupha Pokhari at around 3000 m altitude (n=29). The PFT was recorded using digital spirometer (Chestgraph, HI-101, Japan) and achieved in the department of Basic and Clinical Physiology, BPKIHS. Subjects with any disease or on any medication were excluded from the study. Data were extracted and analyzed using descriptive statistics. Correlation between spirometric parameters and

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demographic parameters were done using Pearson's correlation. The P<0.05 was considered as statistical significance.
Results: All included data were from male healthy native highlanders; age 28.26±7.19 years, body mass index 24±3.64 kg/m^2, body surface area 1.9±0.11 m^2. Spirometric parameters were; TV 0.54±0.06 L, IRV 2.68±0.59 L, ERV 0.86±0.43 L, FVC 4.17±0.57 L, FEV1 3.9±0.54 L, FEV1/FVC % 93.9±5.1, and MVV 90.81±31.29 L. Only VC showed negative correlation with age. Other spirometric parameters did not show any relationship with demographic parameters.
Conclusion: All spirometric data were from healthy male native highlanders in adult age group. Only vital capacity (VC) showed negative correlation with age. Other spirometric parameters did not show any relationship with any of the demographic parameters. These data establish pulmonary function test/spirometric data of healthy male high-altitude dwellers in eastern Nepal for the first time in Nepal.

Keywords: highlanders, pulmonary function test, spirometric, Gupha Pokhari

Poster

P5-1

Antidiabetic drug omarigliptin induces vasodilation via Kv channels and SERCA pump activation in rabbit aorta

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We investigated the vasodilatory effect of omarigliptin, an oral antidiabetic drug in the dipeptidyl peptidase-4 inhibitor class, and its related mechanisms using phenylephrine (Phe)-induced pre-contracted aortic rings. Omarigliptin dilated aortic rings pre-constricted with Phe in a dose-dependent manner. Pretreatment with the voltage-dependent K^+ channel inhibitor 4-aminopyridine significantly attenuated the vasodilatory effect of omarigliptin, whereas pretreatment with the inwardly rectifying K^+ channel inhibitor Ba^{2+}, ATP-sensitive K^+ channel inhibitor glibenclamide, and large-conductance Ca^{2+}-activated K^+ channel inhibitor paxilline did not alter its vasodilation. Pretreatment with the sarco-/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pump inhibitors thapsigargin and cyclopiazonic acid significantly reduced the vasodilatory effect of omarigliptin. Neither cAMP/PKA-related signaling pathway inhibitors nor cGMP/PKG-related signaling pathway inhibitors modulated the vasodilatory effect of omarigliptin. Removal of endothelium did not diminish the vasodilatory effect of omarigliptin. Furthermore, pretreatment with the nitric oxide synthase inhibitor L-NAME or small-conductance Ca^{2+}-activated K^+ channel inhibitor apamin, together with the intermediate-conductance Ca^{2+}-activated K^+ channel inhibitor TRAM-34, did not influence the vasodilatory effect of omarigliptin. In conclusion, omarigliptin induced vasodilation in rabbit aortic smooth muscle by activating voltage-dependent K^+ channels and the SERCA pump independently of other K^+ channels, cAMP/PKA- and cGMP/PKG-related signaling pathways, and the endothelium.

Keywords: omarigliptin, voltage-dependent K^+ channel, SERCA pump, Aorta

P5-2

Antimuscarinic drug fesoterodine induces inhibition of voltage-dependent K^+ channels in freshly isolated coronary arterial smooth muscle cells

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Fesoterodine, an antimuscarinic drug, is widely used to treat overactive bladder syndrome. However, there is little information about its effects on vascular K^+ channels. In this study, Kv channel inhibition by fesoterodine was investigated using the patch-clamp technique in rabbit coronary artery. In whole-cell patches, addition of fesoterodine to the bath inhibited the Kv currents in a concentration-dependent manner, with an IC_{50} value of 3.19 ± 0.91 μM and a Hill coefficient of 0.56 ± 0.03. Although the drug did not alter the voltage-dependence of steady-state activation, it shifted the steady-state inactivation curve to a more negative potential, suggesting that fesoterodine affects the voltage-sensor of the Kv channel. Inhibition by fesoterodine was significantly enhanced by repetitive train pulses (1 or 2 Hz). Furthermore, it significantly increased the recovery time constant from inactivation, suggesting that the Kv channel inhibition by fesoterodine is use (state)-dependent. Its inhibitory effect disappeared by pretreatment with a Kv 1.5 inhibitor. However, pretreatment with Kv2.1 or Kv7 inhibitors did not affect the inhibitory effects on Kv channels. Based on these results, we conclude that fesoterodine inhibits vascular Kv channels (mainly the Kv1.5 subtype) in a concentration- and use (state)-dependent manner, independent of muscarinic receptor antagonism.

Keywords: Fesoterodine, Kv potassium channel, coronary artery, Kv1.5 potassium channel, use-dependent

P5-3

Atypical antipsychotic paliperidone induces inhibition of voltage-dependent K^+ currents of rabbit coronary arterial smooth muscle cells

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Paliperidone, an atypical antipsychotic, is widely used to treat schizophrenia. In this study, we explored whether paliperidone inhibited the voltage-dependent K^+ (Kv) channels of rabbit coronary arterial smooth muscle cells. Paliperidone reduced Kv channel activity in a concentration-dependent manner with a half-maximal inhibitory concentration (IC_{50}) of 16.58 ± 3.03 μM and a Hill coefficient of 0.60 ± 0.04. It did not significantly shift the steady-state activation or inactivation curves, suggesting that the drug did not affect the gating properties of Kv channels. In the presence of paliperidone, application of 20 repetitive depolarizing pulses at 1 and 2 Hz gradually increased inhibition of the Kv current. Further, the recovery time constant after Kv channel inactivation was increased by paliperidone, indicating that it inhibited the Kv channel in a use- (state)-dependent manner. Its inhibitory effects were reduced by pretreatment with a Kv1.5 subtype inhibitor. However, pretreatment with a Kv2.1 or Kv7 inhibitor did not reduce its inhibitory effect. We conclude that paliperidone inhibits Kv channels (mainly Kv1.5 subtype channels) in a concentration- and use (state)-dependent manner without changing channel gating.

Keywords: Paliperidone, voltage-dependent K+ channel, coronary arterial smooth muscle, use-dependent, Kv1.5 subtype

P5-4

Vasorelaxant mechanisms of the antidiabetic anagliptin in rabbit aorta: roles of Kv channels and SERCA pump

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The present study investigated the vasorelaxant mechanisms of an oral antidiabetic drug, anagliptin, using phenylephrine (Phe)-induced pre-contracted rabbit aortic rings. Arterial tone measurement was performed in rabbit thoracic aortic rings. Anagliptin induced vasorelaxation in a dose-dependent manner. Pre-treatment with the classical voltage-dependent K^+ (Kv) channel inhibitors 4-aminopyridine and tetraethylammonium significantly decreased the vasorelaxant effect of anagliptin, whereas pre-treatment with the inwardly rectifying K^+ (Kir) channel inhibitor Ba^{2+}, the ATP-sensitive K^+ (K_{ATP}) channel inhibitor glibenclamide, and the large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channel inhibitor paxilline did not attenuate the vasorelaxant effect. Furthermore, the vasorelaxant response of anagliptin was effectively inhibited by pre-treatment with the sarco-/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pump inhibitors thapsigargin and cyclopiazonic acid. Neither cAMP/protein kinase A (PKA)-related signaling pathway inhibitors (adenylyl cyclase inhibitor SQ 22536 and PKA inhibitor KT 5720) nor cGMP/protein kinase G (PKG)-related signaling pathway inhibitors (guanylyl cyclase inhibitor ODQ and PKG inhibitor KT 5823) reduced the vasorelaxant effect of anagliptin. Similarly, the anagliptin-induced vasorelaxation was independent of the endothelium. Based on these results, we suggest that anagliptin induced vasorelaxation in rabbit aortic smooth muscle by activating Kv channels and the SERCA pump independent of other vascular K^+ channels, cAMP/PKA- or cGMP/PKG-related signaling pathways, and the endothelium.

Keywords: Anagliptin, voltage-dependent K^+ channel, SERCA pump, aorta, vasorelaxation

P5-5

Antipsychotic lurasidone induces inhibition of voltage-gated K^+ channels in coronary arterial smooth muscle cells

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Lurasidone is a second-generation antipsychotic drug used to treat schizophrenia, mania, and bipolar disorder. The drug is an antagonist of the $5\text{-}HT_{2A}$ and D_2 receptors. No effect of lurasidone on the voltage-gated K^+ (Kv) channels has yet been identified. Here, we show that lurasidone inhibits the vascular Kv channels of rabbit coronary arterial smooth muscle cells in a dose-dependent manner with an IC_{50} of 1.88 ± 0.21 μM and a Hill coefficient of 0.98 ± 0.09. Although lurasidone (3 μM) did not affect the activation kinetics, the drug negatively shifted the inactivation curve, suggesting that the drug interacted with the voltage sensors of Kv channels. Application of 1 or 2 Hz train steps in the presence of lurasidone significantly increased Kv current inhibition. The recovery time after channel inactivation increased in the presence of lurasidone. These results suggest that the inhibitory action of lurasidone is use (state)-dependent. Pretreatment with a Kv 1.5 subtype inhibitor effectively reduced the inhibitory effect of lurasidone. However, the inhibitory effect on Kv channels did not markedly change after pretreatment with a Kv 2.1 or a Kv7 subtype inhibitor. In summary, lurasidone inhibits vascular Kv channels (primarily the Kv1.5 subtype) in a concentration- and use (state)-dependent manner by shifting the steady-state inactivation curve.

Keywords: lurasidone, voltage-gated K^+ channel, electrophysiology, coronary arterial smooth muscle cell

P5-6

A muscarinic acetylcholine receptor inhibitor benztropine blocks the voltage-dependent K^+ channels in rabbit coronary arterial smooth muscle cells

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We investigated the effect of the acetylcholine muscarinic receptor inhibitor benztropine on voltage-dependent K^+ (Kv) channels in rabbit coronary arterial smooth muscle cells. Benztropine inhibited Kv currents in a concentration-dependent manner, with an apparent IC_{50} value of 6.11 ± 0.80 μM and Hill coefficient of 0.62 ± 0.03. Benztropine shifted the steady-state activation curves toward a more positive potential, and the steady-state inactivation curves toward a more negative potential, suggesting that benztropine inhibited Kv channels by affecting the channel voltage sensor. Train pulse (1 or 2 Hz)-induced Kv currents were effectively reduced by the benztropine treatment. Furthermore, recovery time constants of Kv current inactivation increased significantly in response to benztropine. These results suggest that benztropine inhibited vascular Kv channels in a use (state)-dependent manner. The inhibitory effect of benztropine was canceled by pretreatment with the Kv 1.5 inhibitor, but there was no obvious change after pretreatment with Kv 2.1 or Kv7 inhibitors. In conclusion, benztropine inhibited the Kv current in a concentration- and use (state)-dependent manner. Inhibition of the Kv channels by benztropine primarily involved the Kv1.5 subtype.

Keywords: benztropine, voltage-dependent K^+ channel, coronary arterial smooth muscle, use-dependent

P5-7

The vasodilatory mechanisms of DPP-4 anti-diabetic drug trelagliptin in rabbit aorta

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We investigated the vasodilatory effects of trelagliptin and its related mechanisms using rabbit aortic rings. Trelagliptin induced vasodilation in a dose-dependent manner. Pretreatment with the ATP-sensitive

K+ channel inhibitor, large-conductance Ca^{2+}-activated K^+ channel inhibitor, and inwardly rectifying K^+ channel inhibitor did not affect the vasodilatory effect of trelagliptin. However, pretreatment with the voltage-dependent K^+ (Kv) channel inhibitors significantly attenuated the vasodilatory effect of trelagliptin, suggesting that the vasodilatory effect of trelagliptin is associated with Kv channel activation. Although pretreatment with Kv1.5 and Kv2.1 subtype inhibitors did not affect the response to trelagliptin, pretreatment with a Kv7.X subtype inhibitor effectively reduced the vasodilatory effect of trelagliptin. Furthermore, SERCA pump inhibitors also significantly attenuated the vasodilatory effect of trelagliptin. These effects, however, were not affected by pretreatment with Ca^{2+} channel inhibitors, adenylyl cyclase/PKA inhibitors, guanylyl cyclase/PKG inhibitors, or removal of the endothelium. From these results, we concluded that the vasodilatory effect of trelagliptin was associated with the activation of Kv channels (primary the Kv7.X subtype) and SERCA pump.

Keywords: Trelagliptin, voltage-dependent K^+ channel, SERCA pump, Aorta

Poster

P5-8

Encainide, a class Ic anti-arrhythmic agent, blocks voltage-dependent potassium channels in coronary artery smooth muscle cells

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Voltage-dependent K^+ (Kv) channels are widely expressed on vascular smooth muscle cells and regulate vascular tone. Here, we explored the inhibitory effect of encainide, a class Ic anti-arrhythmic agent, on Kv channels of vascular smooth muscle from rabbit coronary arteries. Encainide inhibited Kv channels in a concentration-dependent manner with an IC_{50} value of 8.91 ± 1.75 μM and Hill coefficient of 0.72 ± 0.06. The application of encainide shifted the activation curve toward a more positive potential without modifying the inactivation curve, suggesting that encainide inhibited Kv channels by altering the gating property of channel activation. The inhibition by encainide was not significantly affected by train pulses (1 and 2 Hz), indicating that the inhibition is not use (state)-dependent. The inhibitory effect of encainide was reduced by pretreatment with the Kv1.5 subtype inhibitor. However, pretreatment with the Kv2.1 subtype inhibitor did not alter the inhibitory effects of encainide on Kv currents. Based on these results, encainide inhibits vascular Kv channels in a concentration-dependent and use (state)-independent manner by altering the voltage sensor of the channels. Furthermore, Kv1.5 is the main Kv subtype involved in the effect of encainide.

Keywords: Encainide, voltage-dependent K^+ channel, coronary artery, activation curve, inactivation curve

P5-9

Immature cell-cell contact and increased activity of store-operated Ca^{2+} entry in senescent vascular endothelial cells

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Background: Vascular endothelial cells acquire functionally differentiated phenotype upon maturation of cell-cell contact, thereby regulating vascular permeability and homeostasis. Disruption of barrier function plays a crucial role in the early phase of development of various vascular diseases. Better understanding the molecular basis for maintenance of barrier integrity and its disruption is critical for preventing vascular diseases. Intracellular Ca^{2+} signaling mediated mainly by store-operated Ca^{2+} entry (SOCE) mechanism underlies barrier disruption. Since aging is one of important factors that predisposes various vascular diseases, the present study investigated the effect of replicative senescence on the maturity of cell-cell contact and the activity of SOCE in cultured porcine aortic endothelial cells (PAEC).
Main observations: In PAEC at passages 9-15 and at confluence (Day 7), 1 U/mL thrombin decreased trans-endothelial electrical resistance and induced di-phosphorylation of myosin light chain (ppMLC) and actin bundle formation at cell periphery at 3 min, which was then reorganized to stress fibers in the later phase. In PAEC at early culture day (Day 3) or in the confluent PAEC exposed to Ca^{2+}-free media, thrombin induced actin stress fiber formation with no transient appearance of peripheral bundles. In confluent PAEC at passages 26-30, some actin stress fibers were observed before thrombin stimulation and further increased after thrombin stimulation. In the absence of extracellular Ca^{2+}, thapsigargin, an inhibitor of endoplasmic Ca^{2+} ATPase, induced a transient elevation of the cytosolic Ca^{2+} concentrations ($[Ca^{2+}]_i$) due to Ca^{2+} release from the intracellular store sites. The following replenishment of extracellular Ca^{2+} induced a sustained elevation of $[Ca^{2+}]_i$ due to SOCE. The $[Ca^{2+}]_i$ elevation due to SOCE in PAEC at higher passages was higher than that observed with PAEC at lower passage. PAEC at higher passages exhibited higher b-galactosidase activity than PAEC at lower passages.
Conclusion: The integrity of cell-cell contact is impaired and the activity of SOCE is augmented in the replicative senescent endothelial cells. These phenotypic changes may underly endothelial dysfunction in the aged population.

Keywords: endothelial cells, aging, calcium signaling, barrier function

P5-10

The connection between resveratrol and early closure of fetal ductus arteriosus

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Background: Ductus arteriosus (DA) is an essential arterial connection between the aorta and the pulmonary artery for fetal circulation. Premature constriction of the ductus arteriosus leads to grossly altered cardiac hemodynamics and intrauterine fetal death. Daily polyphenol intake reduces cardiovascular risk. Polyphenol is thought to have antioxidant, anti-inflammatory, and vasodilator effects. Therefore, in adults, polyphenol intake has been shown to be effective against arteriosclero-

sis and hypertension. On the other hand, previous reports have demonstrated that intake of polyphenol during pregnancy can induce premature constriction of the DA in the fetus. The mechanism of polyphenol-induced premature contraction of the DA in the fetus is not yet shown.
Purpose: We aimed to investigate the mechanism how resveratrol, a polyphenol, intake during pregnancy promoted premature constriction of the DA.
Methods: First, we administered resveratrol to pregnant rats at 50mg/kg, once a day, for four days (from 17 to 20 day) by intraperitoneal administration. We then measured the diameter ratio of the DA to the aorta (Ao) at 20 days with Image J by rapid whole-body freezing method. In addition, we also homogenized maternal placental tissue. Some were used for ELISA to measure PGE_2, and the rest for RNA extraction. Second, we added resveratrol (10, 50, 100μM) to cultured DA smooth muscle cells (DASMCs). After 48 hours of incubation, we collected supernatant fluid and determined hyaluronic acid concentration, and we took RNA from DASMCs. Using qPCR analysis, we determined the expression levels of cyclooxygenase2 (Cox2) (involved in production of prostaglandin E_2) and EP4(prostaglandin receptor type 4 expressed in DA).
Results: We found that maternal administration of resveratrol constricted DA in fetal rats (the DA/Ao ratio was 0.80 in the control group and 0.54 in the resveratrol group. $p<0.0001$). Hyaluronic acid production was decreased in a concentration dependent manner by resveratrol. The qPCR analysis revealed that the expression levels of Cox2 and EP4 mRNAs were significantly decreased in the resveratrol group. And, in placenta tissues, the expression levels of Cox1 and Cox2 mRNAs decreased in the resveratrol group. The ELISA revealed that the PGE_2 concentration in placenta tend to decrease in the resveratrol group.
Conclusion: Our results suggested that maternal administration of resveratrol may cause the premature constriction of the DA by inhibition of PGE_2-EP4 signal.

Keywords: resveratrol, ductus arteriosus constriction, prostaglandin E2, prostaglandin receptor type 4

P5-11

Slower relaxation of pulmonary artery than mesenteric artery in rats and the differential expression of myosin light chain phosphorylation regulating enzymes

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Pulmonary arteries (PA) are exposed to hemodynamic environment and contractile stimuli different from those of systemic arteries. However, differences of PA from systemic arteries regarding their contraction/relaxation parameters and myosin light chain 2 (MLC2) phosphorylation-related signaling proteins are lacking. Here, we investigated the relaxation speed, MLC2 phosphorylation, and the expression levels of soluble guanylate cyclase (sGC), protein kinase G (PKG), Rho-A dependent kinase (ROCK) and myosin light chain phosphatase catalytic subunit (MYPT1) in rat PA and mesenteric arteries (MA). In dual-wire myography study, PA showed slightly wider range of length (diameter) for the active tension in response to 80 mM KCl (80K-contraction). It was notable that PA showed significantly slower relaxation after 80K-contraction. Although pharmacological inhibition of sGC by ODQ treatment slowed the relaxation of MA after 80K-contraction, the impairment of relaxation by ODQ was more prominent in PA. Immunoblot assay showed higher levels of sGC-a and ROCK in PA than MA, while higher expression of sGC-b1 and MYPT1 in MA than PA. Diphosphorylation state of MLC2 (pp-MLC2) is known to be associated with tonic contractile state of arteries, and the ROCK inhibitor treatment decreases pp-MLC2. Interestingly, the level or pp-MLC2 in resting and 80K-treated PA was higher than those of MA. The treatment with ROCK inhibitor (Y27632, 10 mM) could abolish the different of relaxation response between PA and MA. Taken together, we suggest that the higher ROCK and lower MYPT in PA would have induced the higher level of MLC2 phosphorylation, which is responsible for the characteristic slower relaxation in PA than systemic arteries.

Keywords: pulmonary artery, mesenteric artery, sGC/cGMP pathway

P5-12

Enhanced carotid flow-mediated dilation (cFMD) following carotid endarterectomy

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Carotid arteries dilate in response to increased blood flow, associated endothelial shear stress and the release of paracrine hormones such as nitric oxide (NO). As such, carotid flow mediated dilation (cFMD) provides a surrogate index for cerebrovascular function and endothelial health. It is unclear whether carotid endarterectomy (CEA) modifies the endothelium-dependent vasodilator function of the carotid arteries in humans; we therefore assessed vascular function before and after surgery, in comparison to matched controls. Participants (n = 11) aged 72.2 ± 6.7 yr possessed asymptomatic internal carotid artery (ICA) stenosis and underwent CEA. Vascular function was compared to that of apparently healthy control subjects matched for age and sex (CON, n = 15, 73.6 ± 5.4 yr). cFMD was assessed, alongside cerebrovascular reactivity (CVR), in response to a hypercapnia stimulus, before and 4 weeks after surgery. cFMD increased significantly compared to the pre-surgical condition in the CEA group (n = 6, POST: 8.71 ± 3.01% *vs* PRE: 5.48 ± 2.08%, P=0.025). CVR also increased compared to pre-surgical condition in this group (n = 13, POST: 1.85 ± 0.92 *vs* PRE: 1.10 ± 0.67 $cm.s^{-1}.mmHg^{-1}$ $P_{ET}CO_2$), however this change did not achieve statistical significance (P = 0.07). There were no significant changes in the control group for either cFMD or CVR (all P > 0.05). Our findings suggest that CEA enhances carotid artery endothelium-dependent vasodilator function and health in patients with internal carotid stenosis.

Keywords: flow-mediated dilation, carotid stenosis, carotid endarterectomy, endothel, nitric oxide

Poster

P5-13

Relation between shift work and endothelial dysfunction in female health workers in hospital: study on resistin, leukocytes, monocytes, microparticles endothelial and cyclic guanosine monophosphate

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Shift work either on a rotational or permanent is often required to meet the target of company's production, but there is a risk of health problems including inflammation. This study aims to determine whether resistin is an intermediate marker of low-grade chronic inflammatory processes and endothelial dysfunction in shift workers.
This study used a comparative repeated cross-sectional design of female workers in hospitals who worked shifts and non-shifts. Data collection was carried out from June to December 2021 and obtained 272 female workers in the city and district of Bandung. Based on the inclusion criteria, there were 64 shift workers and 54 non-shift workers. Characteristics assessed were not different based on age, nutritional status, blood pressure, physical activity, history of smoking, family planning history, cholesterol, blood sugar, triglycerides, erythrocyte sedimentation rate, PSQI score, DASS score, and nutritional intake. Statistical analysis to distinguish the two groups shift and non-shift was using the Mann Whitney test and for correlation using the Spearman test.
The results showed that there was a significant difference between shift workers and non-shift workers in resistin levels p = 0.004 (1.78 ng/mL vs. 3.33 ng/mL), leukocyte count (p = 0.005; 7182 µL vs. 5900 µL), monocyte count (p = 0.016; 475.45 µL vs. 371.98 µL), the number of CD31+ (p < 0.001; 588.68 u/L vs. 286.44 u/L), the number of CD31+CD62e+ (p = 0.031; 236.16 u/L vs. 185.58 u/L), and levels of cGMP (p <0.01; 8.97 pmol/mL vs. 23.92 pmol/mL). In shift workers, there was a significant difference in the number of CD31+, CD62e+ and CD31+CD62e+ at the first, second, and third examinations (p = 0.006, p = 0.043, p < 0.01). There were significant correlation between resistin and the mean CD31+CD62e+ p = 0.024 r = 0.284, resistin with levels of CD31+CD62e+ after night shift p = 0.014 r = 0.345.
It was concluded that resistin can be used as an intermediary for the occurrence of endothelial dysfunction which is characterized by a decrease in GMFs and an increase in the number of CD31+ in shift workers.

Keywords: cardiovascular, cGMP, inflammation, microparticles, Resistin

P5-14

The forgotten circulation: sympathetic control of mesenteric venous capacity in conscious hypertensive rats

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The venous circulation is often "forgotten", as cardiovascular research and therapeutic intervention focusses predominantly on arteries. However, systemic veins store two-thirds of blood volume at rest, with reservoir beds such as the mesenteric circulation receiving dense sympathetic innervation, suggesting a critical neurogenic role for the mobilization of blood. The impact of hypertension on sympathetic regulation of venous circulation remains unclear. We hypothesized that capacity of mesenteric vasculature is impaired in spontaneously hypertensive rats (SHR) and that reducing sympathetic drive will increase mesenteric buffering of excess volume.
SHR (n=6) and Wistar rats (n=7) were instrumented with telemeters recording arterial and venous pressures, and an atrial balloon for assessment of mean circulatory filling pressure (MCFP). Arterial (inflow) and venous (outflow) mesenteric flow probes were implanted to measure total mesenteric volume fluctuation. Mesenteric vascular capacity was challenged with 20% volume load of intravenous saline. In SHR sympathetic drive was reduced via hexamethonium (10mg/kg) or via carotid body denervation (CBD).
MCFP was elevated in SHR (+2.7±0.5mmHg, p<0.01 vs Wistars). MCFP in SHR was reduced by hexamethonium (-2.6±0.6; p=0.01 vs Baseline, n=5) and CBD (–2.0±0.7mmHg; p=0.04 vs Baseline, n=5). In normotensives volume load was accommodated by the mesenteric bed, with a net total influx of blood (+5.4±2.2ml; n=7). In contrast, SHR showed a counterintuitive net efflux of blood from the mesenteric bed (-1.6±1.4ml; p=0.04 vs Wistars, n=6). Preliminary results indicate that hexamethonium and CBD tended to increase the ability of the mesenteric bed to accommodate volume (hexamethonium: +1.9±1.5, n=6; CBD: +0.8±0.6ml, n=3).
We show that SHR have higher venous tone and reduced capacity to accommodate excess volume within the mesenteric vascular bed. Reductions in sympathetic drive reduced venous tone and improved the ability to accommodate volume load. Inhibiting sympathetic activity may provide a novel therapeutic opportunity to restore venous mesenteric capacity and potentially ameliorate hypertension.

Keywords: venous circulation, sympathetic activity, blood pressure, mean circulatory filling pressure, volume homeostasis

P5-15

Vasodilatory effects of ginsenoside Rg3 against alpha-adrenergic constriction in ovariectomized rats

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Transition to menopause is known to be a risk factor of cardiovascular disease (CVD), which is a worldwide problem. The effect of ginsenosides from ginseng remains controversial for CVD, it has been discovered that Rg3, one of the ginsenosides, has beneficial effects, including anti-inflammatory and enhancing NO production. However, the effect of Rg3 on the systemic vascular system in women is not fully understood. we aimed to investigate the effects of exposure to Rg3 on vascular reac-

tivity in isolated mesenteric arteries (MA) of pre- and postmenopausal rats.
Female Sprague-Dawley rats (165-185 g) were randomly divided into two groups, with a week of acclimatization. At 8 weeks old, the sham group (n=16) received sham surgery, whereas the experimental group (n=16) underwent bilateral ovariectomy (OVX) for postmenopausal rat modeling. They were anesthetized and sacrificed after 4 weeks. The isometric tension of the ring segments from the 3rd branches of the MA was measured using a dual-wire myograph. The vascular reactivity with functional endothelium was tested by phenylephrine (PhE) 10 mM and acetylcholine 10 mM. Endothelial denudation was accomplished by physically disrupting the endothelial cell layer with tungsten wire. The relaxative response to Rg3 (0.05 ~ 10 μM) was applied to each group after α-adrenergic induced vasocontraction with various K^+ channel blockers.
In the OVX group, the maximal constriction in response to both high-K and PhE-induced vasoconstriction significantly increased compared to sham group ($p<0.05$; $p<0.05$). Rg3 induced a dose-dependent vasorelaxant effect on PE pre-constricted vessel with viable endothelium in both sham and OVX groups. Rg3 induced relaxation in endothelium-denuded vessels as well in both groups. In the sham group, the denuded vessel shows significantly lower relaxation than the vessel with endothelium ($p<0.05$), but in the OVX group, the denuded vessel shows no different relaxation than the vessel with endothelium. L-*NG*-Nitro arginine methyl ester (L-NAME) 100 μM incubation with endothelium-intact MA doesn't affect to Rg3 induced vasodilation in both groups. Rg3-induced relaxation was suppressed in both groups in the presence of cocktail of cyclooxygenase inhibitor, small and intermediate conductance Ca^{2+}-activated K^+ channel inhibitor ($p<0.001$). Moreover, voltage-gated K^+ channel inhibitor ($p<0.001$), or large conductance Ca^{2+}-activated K^+ channel inhibitors significantly impaired Rg3-induced relaxation ($p<0.001$).
In conclusion, Rg3 has vasodilation after the α-adrenergic contraction in the sham and OVX groups. Interestingly, the vasorelaxant effect of Rg3 differs depending on the presence or absence of endothelium. Moreover, vasorelaxation in endothelium-denuded vessels is significantly greater in the OVX group than in the sham group. This relaxation of Rg3 was significantly diminished by various K^+ inhibitors and cyclooxygenase inhibitor. Further investigation is required to elucidate the underlying mechanisms of these effects.

Keywords: Rg3, mesenteric artery, ovariectomy, menopause, vascular reactivity

P5-16

Finasteride prevents neointimal hyperplasia and affects vascular smooth muscle cells proliferation, migration, and apoptosis

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Vascular smooth muscle cells (VSMCs) play a key role in the regulation of blood pressure and vascular tone. However, normal functions of VSMCs is disrupted by factors that cause inflammation and oxidative damage to cardiovascular system, such as hypertension, diabetes, hyperlipidemia, and smoking. In this process, VSMCs undergo phenotype switching from contractile to synthetic, and synthetic VSMCs exhibit increased migration and proliferation activity and synthesize and accumulate extracellular matrix. This is part of the repair process, but if excessive, it can lead to neointimal hyperplasia, which narrows the vessel lumen, causing stenosis.
Although the relationship of T and DHT to cardiovascular disease (CVD) remains controversial, T and DHT out of the ideal physiologic range in older men and patients receiving hormone therapy are thought to be a risk factor for CVD. There are also reports of increased CVD risk in women with elevated androgen levels due to polycystic ovary syndrome and menopause. However, studies on the benefits or side effects of anti-androgenic drugs on the cardiovascular system are limited compared to androgens. Therefore, here we investigated the effects of finasteride, a competitive inhibitor of 5α-reductase, on the cardiovascular system and VSMCs.
The effect of finasteride on arterial vascular remodeling was evaluated by inducing neointimal hyperplasia in the left carotid artery of male rats with balloon catheter injury. Following subcutaneous administration of finasteride for 2 weeks, the rats were sacrificed and the arteries were histologically analyzed. In addition, the proliferation, migration, apoptosis and cellular response to finasteride were investigated using primary cultured VSMCs separated from male and female rat thoracic aorta.
As a result, Finasteride can effectively inhibit neointima formation, preventing the narrowing of the blood vessel lumen. Proliferation of primary VSMCs was inhibited by finasteride in both males and females, while migration was inhibited in males but not in females. However, this does not seem to be androgen hormone-dependent response, as finasteride's action was not abolished when VSMCs were co-treated with T and DHT. Furthermore, finasteride induced apoptosis in male and female VSMCs in a concentration-dependent manner. For the first time, we tested the effect of finasteride in neointimal hyperplasia. These results propose a novel cardiovascular action of finasteride and suggest that it can be used as a therapeutic agent to prevent neointimal hyperplasia.

Keywords: neointimal hyperplasia, vascular smooth muscle cell, finasteride

P5-17

Regulation of angiogenic-associated genes by mir-196a-5p in human umbilical vein endothelial cells exposed to hypertensive pregnancies

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Offspring of women with hypertensive disorders of pregnancies (HDP) displayed changes in endothelial function and altered microvascular development from early life. Dysregulated angiogenesis and vascular development in the offspring of HDP may increase their risk of developing cardiovascular diseases in later life. MicroRNAs (miRNAs) are non-coding single-stranded RNA that can regulate gene expression and play a role in modulating angiogenesis and endothelial dysfunction. However, their role in regulating angiogenesis in the endothelial cells of offspring exposed to HDP is not well understood. Therefore, this study aims to identify the differential miRNA expression in endothelial cells derived from the offspring of HDP and their role in regulating angiogenesis markers. The miRNA sequencing was performed on human umbilical vein endothelial cells (HUVEC) isolated from the offspring of HDP to identify the differentially expressed miRNA. Then, the expression of the selected miRNA was validated using stem-loop reverse

transcription quantitative real-time PCR (RT-qPCR). *In silico* analysis via miRWalk, miRDB, TargetScan and DIANA was performed to predict the target genes of the selected miRNA, with emphasis on the genes related to angiogenesis. Transient transfection of HUVEC with the selected miRNA was performed to determine the regulatory role of the selected miRNA on the target genes involved in angiogenesis. miRNA sequencing revealed the differential expression of 218 known miRNAs and 23 unknown miRNAs in HUVEC from HDP compared to the HUVEC from normotensive pregnancies. Of these, miR-196a-5p was significantly upregulated. Further validation with stem-loop RT-qPCR showed upregulation of miR-196a-5p expression by 8-fold in HUVEC from HDP compared to the control ($P < 0.001$). Based on the *in silico* analysis, a total of 96 predicted target genes were identified and subjected to Cytoscape software and DAVID analysis against the Gene Ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases. The analysis revealed that three angiogenic-associated genes (*PDGFRA*, *VEGF* and *bFGF*) were predicted to be targeted by miR-196a-5p. Transient transfection of HUVEC with miR-196a-5p mimic caused significant upregulation of *PDGFRA* ($P <0.0001$), *VEGF* ($P <0.001$) and bFGF ($P <0.0001$) gene expression in HUVEC from HDP, whereas suppression of miR-196a-5p caused upregulation of these genes ($P <0.05$). Collectively, the results indicate that miR-196a-5p may have a regulatory effect on angiogenesis-associated markers and would predict the vasculogenic capacity of endothelial cells in the offspring of HDP.

Keywords: endothelial cells, angiogenesis, mir-196a-5p, hypertensive disorders of pregnancies

Poster

P5-18

The potential mechanism of resistin on endothelial dysfunction through cGMP and endothelial microparticle markers CD 31 and CD 62e

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Resistin is one of the pro-inflammatory substances associated with alterations in sleep patterns and believed to contribute to the development of atherosclerosis due to its ability to induce the production of foam cells. This study aims to found the mechanism of resistin caused endothelial dysfunction that caused of cardiovascular disease in the future on shift worker.

This comparative repeated cross-sectional study was carried out to evaluate resistin values, cGMP levels, and the value of endothelial microparticles in shift and non-shift female health worker at hospitals in Bandung between June to December 2021. The criteria for research subjects were aged between 20-35 years old, at least two years of shift or non-shift work and at least six months of continuous shift work. They were excluded if they were: smoked actively or passively; had BMI greater than 30 kg/m2; had a family history of heart disease; had a history of low or premature birth weight; had chronic infectious diseases, hypertension, or previous heart failure; used inflammatory analgesic drugs for one week, or were pregnant or breastfeeding. The Mann-Whitney test was utilized as a non-parametric test, the Spearman correlation test as a correlation test, and the JASP version 016.2 application as a pathway analysis tool to determine which pathways could affect endothelial dysfunction by cGMP levels and endothelial microparticle values (CD 31 and CD 62e).

Pathway analyzed was to obtain the mechanism that has the greatest influence on endothelial dysfunction. The results were then analyzed to determine the most important mechanistic pathway from proinflammatory resistin, leukocytes, monocytes, and endothelial dysfunction markers, namely cGMP and microparticles, in this case CD31+/CD62e-, CD31-/CD62e+, and CD31+/CD62e+. According to the calculation of categorical regression analysis, shift work can affect by the length of work, MABP, leukocytes, monocytes, resistin, cGMP, and CD31+/CD62e-, with beta coefficients respectively (0.318, 0.236, 0.258, 0.262, -0.033, -0.277 and 0.675). Cyclic guanosin monophosphate, ESR, and triglycerides affected endothelial microparticles represented by CD31+/CD62e+ with path coefficient é = 0.82. ESR, cGMP, and triglycerides were shown to influence 67% of endothelial microparticles. Based on the path coefficient of 0.82 resistin levels have a 74% effect on cGMP, while shift work has a 90.25% effect on resistin levels. Resistin has 49.59% impact on microparticles via the cGMP pathway. However, pathway analysis also provides evidence of this connection since regression analysis demonstrates resistin's role in cGMP levels with $p = 0.01$ and $R = 0.487\%$. It is possible to conclude that resistin has potential mechanism to cause endothelial dysfunction both through the leukocyte and the cGMP pathways due to inhibiting endothelial nitric oxide synthase in endothelial cells and enhancing foam cell formation in macrophages via the cGMP pathway.

Keywords: cGMP, endothelial microparticles, female, shift work, resistin

P5-19

A novel quinazoline derivative exhibits selective vasodilation targeting systemic vasculature with promising hypotensive effect and low hepatotoxicity

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Background: Quinazoline derivatives possessed various pharmacological activities such as anti-inflammation, anti-cancer, anti-erectile dysfunction, phosphodiesterase-5 inhibitory property and anti-hypertension. Recently, we reported that N^2-methyl-N^4-[(thiophen-2-yl)methyl] quinazoline-2,4-diamine (compound 8) has a 5-fold better selectivity for the aorta than the pulmonary artery, indicating its potential utility as a vasodilator of the systemic vasculature. Therefore, this study aimed to investigate the vasorelaxant effect on resistance arteries of the systemic vasculature and the hypotensive effect of compound 8. Its potential impact on hepatocyte viability and cytochrome P450 (CYP) activities were also evaluated.

Methods: The vasorelaxant effects of compound 8 and the underlying mechanisms were investigated using isolated mesenteric arteries of Wistar rats, and the acute hypotensive effect was evaluated in anesthetized rats. Isolated rat hepatocytes were examined for cell viability and

CYP activities. Nifedipine was used as a comparator.
Results: Compound 8 demonstrated a robust vasorelaxant effect similar to that of nifedipine. This effect was endothelium-independent but diminished in the presence of inhibitors targeting guanylate cyclase (ODQ) and K_{Ca} channels (iberiotoxin). Compound 8 enhanced sodium nitroprusside-induced relaxation while inhibiting vasoconstriction mediated by α_1-adrenergic receptor activation and extracellular Ca^{2+} influx via receptor-operated Ca^{2+} channels. Acute intravenous infusion of compound 8 at doses of 0.05 and 0.1 mg/kg resulted in hypotension, demonstrating comparable efficacy to nifedipine in reducing diastolic and mean arterial blood pressure with a lesser impact on systolic blood pressure. Compound 8 did not reduce hepatocyte viability except at concentrations >10 μM and inhibited CYP1A and 3A at 10 μM.
Conclusion: This study identified compound 8 as a powerful vasodilator targeting resistance vessels, resulting in a pronounced acute hypotensive effect while carrying a low risk of liver toxicity or drug-drug interactions. This relaxant effect mainly involved the activation of the sGC/cGMP pathway, the opening of K_{Ca} channels, and the inhibition of Ca^{2+} entry. This compound is a potential lead for developing new drugs for systemic hypertension.

Keywords: quinazoline derivative, vasorelaxation, mesenteric artery, hypotensive effect, hepatotoxicity

P5-20

Opisthenar microvascular area is a better predictor over arterial stiffness of disease severity in acute coronary syndrome patients

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The current study aims to analyze the associations between peripheral microvessel area or arterial stiffness with cardiac function in patients with acute coronary syndrome.
60 hospitalized patients underwent coronary angiography were collected and the degree of coronary arterial stenosis were graded (Gensini score) according to clinical criteria (severity I n=26, severity II n=10, severity III n=12 and control n=12). Cardiac functions and laboratory examination results were compared with opisthenar microvascular area (OMA, ISOCT) and arterial stiffness measurement (PWV, Omron HBP-8000). Inflammatory indexes were used for comparison.
Our results showed that cardiac functions (EF, FS, CO and SV) were negatively associated with the severity of coronary arterial stenosis. OMA were reduced in more severe patients and was negatively associated with coronary arterial stenosis. PWV was not affected by disease severity and no associations were observed between PWV and coronary arterial stenosis. Inflammatory indexes (WBC, monocyte, neutrophil, etc.) were greater in severe patients and positive associations were observed with coronary arterial stenosis. As expected, NLR and MLR were negatively associated with EF and FS ($p < 0.05$); however, OMA or PWV was not linked with cardiac functional parameters.
Our results suggest that peripheral microvascular area may provide better reference value over arterial stiffness for predicting the degree of coronary lesions in ACS patients.

Keywords: coronary arterial stenosis, acute coronary syndrome, peripheral microvascular area, arterial stiffness, inflammation

P5-21

WITHDRAWN

P6-1

Alterations of leptin and metabolism induced by ablation of the bitter taste receptor gene

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Taste is closely related to intake of food. Vertebrate animals recognize bitter taste through type 2 taste receptors (T2Rs). *Tas2r108* of 35 murine t2rs was found to be most highly expressed in various exocrine tissues as well as the tongue. The physiological function of *tas2r108* would be elucidated by pursuing change in plasma leptin (Lep), arterial blood pressure (BP), fasting blood glucose (FBG) and body weight (BW) during 18 month in tas2r108 knock-out mice.
Tas2r108 knock-out mice produced with *CRISPR/Cas9* technology. The expression levels of t2rs were determined by real time PCR. Plasma leptin levels were evaluated by ELISA. BPs were measured by non-invasive technique on tail. FBGs were monitored by Accu-Chek® Instant. Wild type C57BL/6 mice(WT) or tas2r108 knock-out mice(KO) from 8 to 78-week-old were used.
Tas2r108 knock-out does not elicit much change in expression of other tas2rs in taste buds and submandibular glands. From 8 weeks to 78 weeks age, the difference in BPs or FBG between WT and KO mice did not found. The BWs were heavier in KO mice group than WT mice group. The Lep levels higher in KO group than those of WT groups from 12-20 weeks old mice.
The results suggest that at least by 18 months in KO group would elicit change in metabolism or feeding behavior and the change in feeding behavior and/or metabolism in KO group would begin in 12 weeks old mice. However, more continuous research is needed to confirm the exact physiological role of bitter taste.

Keywords: tas2r108, bitter taste, metabolism, feeding behavior, plasma leptin

P6-2

Amphetamine acts through the melanocortin system to regulate metabolism and cardiovascular function

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Background: Amphetamine (AMPH) is well known for its ability to induce substantial and sustained weight loss. In addition to its metabolic effects, AMPH affects the cardiovascular system, increasing blood pressure and heart rate. The central melanocortin system is a key regulator of both metabolic and cardiovascular functions.
Method/Results: This research demonstrates that hypothalamic

proopiomelanocortin (POMC) neurons and the central melanocortin system are required for AMPH-induced anorexia, energy expenditure, tachycardia and hypertension. In diet induced obese (DIO) wild-type mice AMPH excited POMC neurons and significantly increased hypothalamic α-melanocyte stimulating hormone (αMSH) secretion. AMPH reduced the food intake and bodyweight, increased BAT thermogenesis, blood pressure and heart rate. In melanocortin 4 receptor deficient obese (MC4R KO) mice, AMPH significantly increased αMSH secretion although metabolic and cardiovascular effects were significantly attenuated compared to effects in DIO mice. Chronic AMPH treatment in DIO and MC4R KO mice induced significant and sustained weight loss, with cumulative food intake significantly reduced compared to vehicle-treated controls. Despite significant weight loss, blood pressure was elevated in AMPH-treated DIO mice but not in MC4R KO mice. Central serotonergic and noradrenergic systems are responsible for melanocortin-dependent effects of AMPH as antagonism of either or both of these neurotransmitter systems attenuated AMPH-induced αMSH secretion as well as AMPH-induced metabolic and cardiovascular effects.
Conclusion: We propose that AMPH increases serotonergic excitation of POMC neurons, and reduces the noradrenergic inhibition of POMC neurons. These presynaptic mechanisms result in elevated POMC neuron activity, αMSH release and MC4R pathway activation resulting in metabolic and cardiovascular systems outputs. Some of the metabolic action, weight loss of AMPH persist in the absence of MC4R without the adverse cardiovascular actions, this highlights a potential target pathway for future research.

Keywords: amphetamines, MC4R, POMC, blood pressure, food intake

Poster

P6-3

Identifying the role of LETMD1 in maintaining brown adipose tissue adaptive thermogenesis

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Brown adipose tissue (BAT) has high levels of mitochondria and is capable of generating heat via uncoupled respiration. Mitochondria uncoupling protein 1 (UCP1) is activated in BAT during cold exposure and dissipates energy generated from the proton motive force as heat. However, other mitochondrial proteins responsible for BAT adaptive thermogenesis is largely unknown. Here, we identify LETM1 domain-containing protein 1 (LETMD1) as a BAT enriched, cold induced mitochondrial protein that is essential for BAT adaptive thermogenesis during cold exposure. *Letmd1* knockout mice exhibit aberrant mitochondria morphology and function. Oxidative phosphorylation (OXPHOS) complex proteins and UCP1 are deficient in *Letmd1* knockout mice and fail to carry out adaptive thermogenesis. Importantly, BAT specific *Letmd1* knockout mice show similar phenotype as *Letmd1* knockout mice, excluding contributions of other tissues in the phenotype observed in *Letmd1* deficient mice. Lastly, using proximity labeling *in vivo*, we identify LETMD1 as a bona fide mitochondrial matrix protein. Taken together, our findings reveal that LETMD1 is a mitochondrial matrix protein indispensable for cold-stimulated respiration and thermogenic function of BAT.

Keywords: LETMD1, brown adipose tissue, mitochondria, thermogenesis

P6-4

Protein kinase D 1/2 and the scaffold protein Na^+/H^+ exchanger regulatory factor 1 mediate hypoxia-induced *Vegfa* expression in 3T3-L1 adipocytes

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Obesity is characterized by hypertrophy of adipocytes. The expansion of adipose tissue during obesity comes with compromised vascularization and restricted oxygen availability to the enlarged adipocytes, resulting in hypoxia in adipocytes. Hypoxia contributes to dysfunction in adipocytes; however, the mechanism remains incompletely understood. Protein kinase D 1 (PKD1) has been shown to be activated in obese adipocytes. Therefore, we tested if PKD isoforms were involved in hypoxia-induced dysfunction of adipocytes. Hypoxia treatment increased the protein of hypoxia-inducible factor 1α (HIF1α), the key transcription factor orchestrating hypoxia-induced mechanism, in 3T3-L1 adipocytes. Hypoxia treatment also induced PKD phosphorylation in adipocytes, suggesting the activation of PKDs in response to hypoxia treatment. Inhibition or depletion of PKD isoforms attenuated hypoxia-induced increase of HIF1α protein, suggesting the involvement of PKDs in modulating hypoxia-induced mechanism in adipocytes. Hypoxia treatment also induced proinflammatory signaling. However, inhibition of proinflammatory signaling did not affect hypoxia-induced PKD phosphorylation, ruling out PKD activation by elevated proinflammatory signaling. Interestingly, inhibition of PKD isoforms attenuated hypoxia-induced mRNA of *Vegfa*, a target gene of HIF1α, whereas depletion of PKD1 and PKD2 (PKD1/2), but not PKD3, attenuated hypoxia-induced *Vegfa* in adipocytes. PKD1/2 contain a PDZ-binding motif that is not present in PKD3. Previous report identified a scaffold protein Na^+/H^+ exchanger regulatory factor 1 (NHERF1) to interact with the PDZ binding motif of PKD1/2 and to modulate their subcellular localization and activity. We found that hypoxia treatment induced NHERF1 protein and the interaction of NHERF1 with PKD1 in adipocytes. Moreover, depletion of NHERF1 attenuated hypoxia-induced HIF1α protein and *Vegfa* mRNA in adipocytes. Collectively, our results reveal a role of NHERF1-PKD1/2-HIF1α in modulating hypoxia-induced gene expression in adipocytes.

Keywords: hypoxia, PKD, HIF1α, NHERF1, Vegfa

P6-5

Adipocyte copper import via CTR1 is essential for non-shivering thermogenesis

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Brown adipose tissue (BAT) plays a critical role in non-shivering thermogenesis, offering potential benefits for improving metabolic complications associated with obesity and diabetes. Although the presence of copper (Cu) in these tissues is generally thought to be required for adaptive thermogenesis, there is no evidence linking Cu import via the Cu importer CTR1 to adipose thermoregulation. Here, we show that CTR1 protein expression is strongly increased in BAT upon cold exposure, and in white adipose tissue after β3-adrenergic receptor stimulation. In wild type mice, dietary Cu deficiency leads to defective thermogenesis and death upon acute cold exposure. We generated adipocyte-specific Ctr1 knockout ($Ctr1^{ad/ad}$) mice, which exhibit systemic metabolic abnormalities. Acute cold exposure evokes a severe hypothermia in $Ctr1^{ad/ad}$ mice resulting in the death of these mice in several hours. Furthermore,

cold-induced lipid depletion in the BAT of $Ctr1^{ad/ad}$ mice is significantly impaired as compared to that in control mice, suggesting that CTR1-mediated Cu import is required for lipolysis-mediated BAT activation. Notably, elesclomol (ES) prevents cold-induced hypothermia in $Ctr1^{ad/ad}$ mice through its effects on Complex IV expression and lipolysis-mediated BAT activation. In summary, these studies identify CTR1-mediated Cu import in adipose tissue as a mechanism that occurs Cu-dependent thermogenesis and lipolysis-mediated BAT activation, which offer potential therapeutic strategies for addressing metabolic disorders.

Keywords: Brown adipose tissue, Copper, Ctr1, energy metabolism, thermogenesis

P6-6

Ginsenoside compound K promotes thermogenic signature of white adipose via mitochondrial dynamics and biogenesis

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The ginsenoside CK is a metabolite converted from Rb1 by the gut microbiota and is considered a major active constituent of ginseng, which has been shown to have anti-inflammatory, anticancer, anti-diabetic, and anti-obesity effects. However, there are no reports on whether CK is involved in the induction of tissue remodeling and mitochondrial biosynthesis in white adipocytes. Therefore, in this study, we investigated the effects of CK on thermogenesis and mitochondrial biosynthesis by treating cold-exposed mice and mouse iWAT stromal vascular fraction (SVF) cells with CK. CK promoted the expression of UCP1 and other brown and beige adipocyte/fat factors (Prdm16, Pgc1α, Cd137, Tbx1, Tbp1, Cytb, Uqcrc1, Letm1) and mitochondrial biogenesis and dynamics related factors (Cidea, Cox8b, Fgf21, Cycs, Dio2, Sirt3, Nrf1, Tfam, Drp1, Fis1) in 3T3-L1/iWAT SVF cells, leading to the transdifferentiation of white adipocytes into beige adipocytes. These browning effects of CK were abolished by knockdown of Mdivi-1, a potent DRP1 inhibitor, or SIRT3. These results suggest that CK induces beige remodeling of WAT by regulating mitochondrial dynamics and SIRT3.

Keywords: ginsenoside CK, thermogenesis, mitochondria dynamics, mitochondria biogenesis

P6-7

AdipoArea software based on deep learning techniques improving the precision of adipocyte size assessment

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The size of adipocytes is related to obesity, and changes in the number and size of adipocytes, relative to total body fat, play an important role in the development and progression of obesity. Additionally, the size of adipocytes is known to be associated with various metabolic disorders. Therefore, the development of an accurate method for measuring adipocyte size can provide useful information for the prevention and treatment of obesity-related diseases, maintenance of healthy metabolic function, and more. Currently, there are methods for measuring adipocyte size in tissue samples using image analysis technology, but there is still room for improvement in terms of accuracy and efficiency. Therefore, a more accurate and efficient method for measuring adipocyte size can greatly benefit research and treatment of the association between obesity and metabolic disorders. In this study, we introduce a new adipocyte measuring program, named AdipoArea. This program uses deep-learning-based algorithm to measure the sizes of cells and provides higher accuracy than previously reported conventional adipocyte counting programs in the measurement of adipocytes. It can be used as a supporting tool to correct errors in conventional fully automated adipose cell counting methods, or as an end-to-end program to measure adipocyte area or size. Using this software, the researchers can reduce the contamination of nonspecific elements of relatively small size while providing more accurate data analysis values based on a low error rate in drug or mechanism research that controls the size of adipocytes by using the AdipoArea software.

Keywords: adipocytes, obesity, measurement, deep learning, software

P6-8

GDF15 regulates fat metabolism and induces foamy macrophage in adipose tissue in chronic alcohol consumption

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Background: Alcohol-related liver disease (ALD) accounts for the second highest proportion among chronic liver diseases, following chronic hepatitis B. Among the various organs involved, adipose tissue (AT) plays a significant role in ALD by releasing free fatty acids (FFAs) into the liver, thereby contributing to liver damage. Growth and differentiation factor 15 (GDF15), a cytokine involved in inter-organ communication, is known for its regulatory role in macrophage activation and lipid metabolism in AT. However, it remains uncertain whether GDF15 also modulates fat metabolism in adipose tissue macrophages (ATMs) in individuals with ALD.
Methods: To investigate the impact of GDF15 on ATMs in ALD, a study was conducted using wild-type (WT) mice and mice with hepatocyte-specific GDF15 knock-out (cKO). These mice were fed a Lieber-DeCarli liquid alcohol diet (4.5%) for a duration of 8 weeks. Liver and AT samples were collected and subjected to biochemical, histopathological, and bulk RNA-sequencing analyses. In addition, in vitro experiments were performed using bone marrow-derived macrophages (BMDM) cultured and treated with various chemicals for flow cytometry, BODIPY staining, and qRT-PCR analyses.
Results: In WT mice subjected to chronic alcohol consumption, hepatic GDF15 expression was upregulated while GDF15 expression in AT was downregulated, coinciding with an increase in foamy macrophages in AT. Bulk RNA-sequencing and qRT-PCR analyses demonstrated that alcohol intake suppressed GDF15 expression but upregulated the expression of lipolysis-related genes and colony-stimulating factor 1 (CSF1) in AT. *In vitro* experiments conducted on BMDMs revealed that co-treatment of GDF15 and CSF1 induced morphological changes and promoted fat accumulation, as confirmed by BODIPY staining. GDF15 treatment increased fatty acid uptake genes and decreased CPT1 expression, reducing mitochondrial ROS production in BMDMs. These findings suggest that GDF15 might regulate fat storage and fatty acid

oxidation in ATMs, potentially contributing to the reduction of serum FFA levels, especially in ALD.
Conclusion: Our study demonstrates that hepatic GDF15 is involved in the regulation of cytoskeletal structures in ATMs, facilitating fat storage. Furthermore, by inhibiting the activation of these macrophages through fat oxidation, GDF15 effectively mitigates ALD. Therefore, targeting GDF15 or its downstream signaling pathways may provide potential therapeutic targets for the treatment of ALD.

Keywords: alcohol-associated liver disease, adipose tissue, growth/differentiation factor 15, foamy macrophage, free fatty acid

P6-9

RNA binding protein HuR is essential for adaptive thermogenesis

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Brown adipose tissue (BAT) plays a key role in the regulated production of heat in response to cold stimulus, known as adaptive thermogenesis. While the transcriptional mechanisms underlying the thermogenic function of BAT has been extensively studied, the contribution of post-transcriptional processes to this process remains largely unexplored. RNA binding proteins are known to play a central in controlling multiple aspects of posttranscriptional RNA biology. In this study, we focused on understanding the role of the RNA binding protein HuR in the regulation of BAT thermogenic function. To investigate this, we generated mice with adipocyte-specific deletion of HuR (HuR^{AKO}) and found that these mice were unable to increase energy expenditure in response to a cold challenge, resulting in marked hypothermia. HuR^{AKO} mice housed at thermoneutrality also failed to increase energy expenditure upon β3 adrenoreceptor agonist treatment, indicating a primary defect in thermogenic BAT function. Further examination of brown adipocytes lacking HuR revealed impaired mitochondrial respiration under β-adrenergic stimulation. RNA-seq analysis of HuR^{AKO} BAT revealed that loss-of-HuR led to downregulation of genes associated with metabolic pathways including TCA cycle, β-oxidation, and mTORC1 signaling. Importantly, we observed a partial rescue of hypothermia in HuR^{AKO} mice upon pharmacological activation of mTORC1, suggesting that mTORC1 signaling functions downstream of HuR to mediate brown fat thermogenesis. Our findings reveal an essential role for RNA binding protein HuR in thermogenic programs of the brown adipocyte and highlight the importance of posttranscriptional processes in brown fat thermogenesis.

Keywords: brown adipose tissue, adaptive thermogenesis, post-transcriptional regulation, RNA binding protein, Human antigen R

P6-10

Inhibition of lactate dehydrogenase a stimulates lipid catabolism and thermogenesis via AMPK activation in mouse brown adipose tissue

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Lactate dehydrogenase (LDH) A and B are key regulators of glycolysis and catalyze the reversible conversion between pyruvate and lactate. Recent studies have shown that lactate can upregulate mitochondrial function and thermogenesis in brown adipose tissue. However, the regulatory roles of LDH in adipose tissue metabolism have not been thoroughly investigated. In this study, we demonstrated that LDH-A is predominantly expressed in mouse brown adipose tissue. Genetic suppression of LDH-A increased mitochondrial protein expression, including uncoupling protein 1 (UCP1) and electron transport chain, and inhibited glycolysis in differentiated brown adipocytes. Declined ATP content by LDH-A suppression stimulated AMP-activated kinase (AMPK) signaling, leading to upregulation of peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC1α) and attenuation of lipogenesis via acetyl-CoA carboxylase inactivation. These metabolic alterations were blocked by compound C, an inhibitor of AMPK. Sodium oxamate, a competitive inhibitor of LDH-A, also upregulated mitochondrial proteins, raised NADH/NAD ratio, inhibited glycolysis, depleted ATP content and activated AMPK in brown adipocytes. Additionally, oxamate increased proton leak, indicating enhanced UCP1 function. Injection of oxamate into normal chow and high-fat diet mice decreased lipid droplet size and number in the liver and adipose tissues and increased thermogenesis in vivo. In conclusion, our findings suggest that LDH could be a novel and promising therapeutic target for obesity and chronic metabolic diseases through improving thermogenesis and lipid catabolism.

Keywords: lactate dehydrogenase A, AMP-activated kinase, uncoupling protein 1, thermogenesis, brown adipocyte

P6-11

Tracking of human adipocyte differentiation process and lipid-droplet quantification using low-coherence holotomography

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Visualization and time-lapse tracking of adipocytes and their lipid droplets (LDs) during the differentiation process is challenging due to the long-term differentiation period. The traditional fat staining method is unable to observe the whole differentiation process over a month. Holotomography (HT) has emerged as a useful tool for long-term observation and imaging covered by the entire adipogenesis process and analysis of adipocytes without additional staining and fixation.
In this study, we applied a low-coherence holotomography imaging

system to monitor the redifferentiation process of dedifferentiated human adipocytes for 42 days. Images of live adipocytes were acquired every three days in the same region, and the continuously increasing LD contents were quantitatively measured. The LDs were segmented by the refractive index (RI) thresholding method, and we demonstrated that the analysis of LDs using RI is reasonable through fluorescence staining of LDs. The volume, projected area, and dry mass of LDs gradually increased as adipocyte maturation progressed. The results suggest that low-coherence holotomography is an effective tool for monitoring live adipocytes differentiation process and analyzing LD accumulation. Overall, this study provides insights into a new approach in adipogenesis and lipid studies using HT and image-based analysis.

Keywords: adipocyte redifferentiation, time-lapse tracking of adipocytes, human adipocyte differentiation, lipid-droplet quantification, holotomography

P6-12

AAV-mediated Fndc5 gene therapy targeting skeletal muscle does not facilitate iWAT browning in mice

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There is increasing knowledge on the beneficial effects of irisin in different organ systems, including adipose, liver, bone, and brain. As a myokine secreted by the muscles after exercise, irisin's effects in the inguinal white adipose tissue (iWAT) to switch into beige adipocytes in a molecular event called "browning" is of great interest to the obese population. Recent advances in gene therapy have acknowledged adeno-associated virus (AAV) as promising vectors for gene delivery in treating various human diseases, but none yet for directly managing obesity. Thus, delivery of irisin to the muscles, as its precursor FNDC5 gene, has become an attractive subject. In this study, we injected mice expressing muscle-specific Cre recombinase (HSA cre) with adeno-associated virus that contains a double-floxed inverse orientation (DIO) element for loxP recognition sites, the irisin precursor Fndc5, and a gfp reporter gene. After confirmation of irisin overexpression in the skeletal muscle, we measured the effects of muscle-expressed irisin especially on the iWAT. We also observed the effects of secreted irisin in the adjacent bones (tibia and femur), with evidences of bone remodeling. High-fat diet (HFD)-fed HSA cre mice showed significantly increased expression of some thermogenic genes after irisin overexpression. However, there were no significant changes in body weight and no browning effect in the iWAT.

Keywords: irisin, myokine, inguinal white adipose tissue, browning, adeno-associated virus

P6-13

Improvement of acetylsalicylic acid on high level glucose induced damage in pancreatic beta cells

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Background: Diabetes is becoming a serious threat to human health worldwide and has affected quality of life in Taiwan. Acetylsalicylic acid (ASA, also known as aspirin) is a commonly used drug for preventing cardiovascular disease in patients with type 2 diabetes mellitus in Taiwan. However, the effect of ASA on the reduction of the defection of beta cells remains unclear. Based on an increase in β-cell metabolism may drive diabetes progression. Therefore, in this study, we evaluated the effect of ASA on damage in the pancreatic êžµ cell (INS-1 cell) of rats.
Methods: The protective effects of ASA on the INS-1 cell line was evaluated following the exposure of the cells to normal glucose level (11 mM) or high glucose level (33 mM) to mimic normoglycemia and hyperglycemia situations, respectively. Possible action mechanisms were determined by analyzing the generation of reactive oxygen species (ROS), nitrites, and lactate dehydrogenase (LDH). All test results are presented in terms of the mean ± SEM. Differences in mean values among groups were analyzed using one-way analysis of variance and a least significant difference post hoc analysis in SPSS Statistics version 22. Statistical significance was indicated if $P < 0.05$.
Results: Although the effect of ASA was not significant in the normal glucose condition, ASA significantly reversed the elevation of ROS, nitrites production, and LDH levels induced by high glucose in INS-1 cells. The results revealed that only under high-glucose conditions did ASA reduce the oxidative stress and cytotoxicity in INS-1 cells. This finding may explain, at least partially, the protective effect of the clinical use of ASA in patients with diabetic mellitus.
Conclusion: ASA results in anti-cell damage under hyperglycemic conditions through a decrease in LDH levels. However, additional studies using diabetes models are required to validate this finding and investigate the underlying mechanism. Furthermore, the manipulation of intracellular LDH levels in β-cells are a potential therapeutic focus for hyperglycemia. Moreover, LDH level is a potential marker of β-cell damage.

Keywords: acetylsalicylic acid, pancreatic beta cells, high level glucose, diabetes, aspirin

P6-14

Effects of fatty acid exposure on insulin secretion and role of Nrf2 in pancreatic beta cells

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Background: In type 2 diabetes, glucose-stimulated insulin secretion (GSIS) from pancreatic β-cells is impaired. Impaired GSIS is caused by oxidative stress due to excessive production of reactive oxygen species (ROS) associated with glucotoxicity and lipotoxicity; however, the detailed mechanism has not been clear. Nuclear factor erythroid 2-related factor 2 (Nrf2), a transcription factor that regulates genes related to the antioxidants, binds to its cytosolic regulatory factors such as Kelch-like

ECH-associated protein 1 (Keap1) under nonstress conditions. Under oxidative stress, Nrf2 is released from the binding of Keap1 and translocated into the nucleus. Activation of Nrf2 in β-cells exposed to oxidative stress under diabetic conditions has been reported to increase cell viability. In the present study, we investigated the involvement of Nrf2 on insulin secretory function in β-cells.
Methods: Rat pancreatic beta cell lines (INS-1 cells) were cultured in RPMI 1640 medium with or without 0.5 mM palmitate for 48 hrs before performing the following experiments. On the other hand, Nrf2 siRNA, Keap1 siRNA or Universal Negative Control siRNA were transfected into INS-1 cells using Lipofectamine RNAiMAX reagent 48 hrs prior to performing the following experiments. The treated cells were pre-incubated for 60 min in the buffer with 2 mM glucose (basal level) and then incubated for 60 min in the buffer containing various concentrations of glucose. Aliquots of supernatant from the incubation buffer were subjected to insulin ELISA. The cells after incubation were subjected to measurement of intracellular ROS, NADPH, ATP, mRNA levels were measured by RT-qPCR, and protein levels were measured western blot.
Results: Palmitate exposure for 48 hrs to INS-1 cells decreased insulin secretion and increased intracellular ROS levels under high glucose stimulation. The decrease in GSIS and the increase in intracellular ROS levels by palmitate exposure were completely restored to the level of unexposure of palmitate by ROS scavengers (ascorbic acid + α-tocopherol). Palmitate exposure for 6~48 hrs decreased Nrf2 mRNA levels and for 12 hrs Keap1 mRNA levels compared with palmitate unexposure. Palmitate exposure for 24 and 48 hrs decreased Nrf2 protein levels. Nrf2 knockdown in INS-1 cells decreased GSIS and increased intracellular ROS levels. Keap1 knockdown increased GSIS. The decrease in GSIS by Nrf2 deficiency was only partially restored by ROS scavengers, while the increase in intracellular ROS levels by Nrf2 deficiency was completely recovered by ROS scavengers. Nrf2 knockdown tended to decrease NADPH levels and intracellular ATP levels under high glucose stimulation. Keap1 knockdown increased intracellular ATP levels.
Conclusion: These results indicate that decreased GSIS under lipotoxicity is attributed to increased ROS and suggests that the reduction of Nrf2 expression is involved in increased ROS. In addition, Nrf2 may regulate GSIS by other mechanisms as well as modulating antioxidant activity.

Keywords: insulin secretion, Nrf2, Keap1, oxidative stress, palmitate

Poster

P6-15

The impacts of NPFFR2 deletion on obesity-induced insulin resistance and metabolic symptoms

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As the physiological receptor of NPFF, NPFFR2 is highly expressed in the hypothalamus and known to be involved in energy homeostasis. Previous studies have demonstrated the involvement of NPFF-NPFFR2 system in controlling feed consumption, glucose metabolism, and diet-induced thermogenesis. In the current studies, we investigate the impacts of NPFFR2 deletion on insulin resistance and metabolic symptoms in diet-induced obese mice. The conventional NPFFR2 knockout (KO) mice and corresponding wild-type (WT) mice were fed with high-fat high-sucrose diet (HFSD) for 25 weeks to induce obesity and its related metabolic dysregulations. A reduction of food consumption and reduced body weight gain were noticed in NPFFR2 KO mice, along with improved circulating lipid parameters like triglycerides and non-esterified fatty acids. There was no difference in total cholesterol level between WT and NPFFR2 KO mice. Transcriptional levels of hypothalamic orexgenic peptides AgRP and NPY were significantly lower in NPFFR2 KO mice compared to WT controls. Glucose homeostasis was evaluated in those mice. By intravenous injection of insulin, insulin-induced AKT phosphorylation was measured in mediobasal hypothalamus, liver and muscle via western blotting. Higher levels of phosphorylated AKT ser473 in the hypothalamus NPFFR2 KO mice indicated the amelioration of obesity-triggered central insulin resistance after NPFFR2 deletion. Similar results were found in muscle tissues. However, NPFFR2 KO mice exhibited increased serum insulin levels and HOMA-IR compared to WT mice. The results of the glucose tolerance test also showed deteriorated glucose intolerance after NPFFR2 deletion. These findings suggest distinct regulatory mechanisms of NPFFR2 in central and peripheral regions. Overall, our results provide evidence that NPFFR2 knockout improves metabolic symptoms and enhances central insulin sensitivity associated with diet-induced obesity.

Keywords: NPFF, NPFFR2, obesity, insulin resistance

P6-16

Liver receptor homolog-1 mediated mechanism of cystathionine gamma-lyase in the liver

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Liver receptor homolog-1 (LRH-1) is a member of the nuclear receptor NR5A subfamily. It is expressed in tissues derived from endodermal origin, liver. It plays a crucial role in bile acid synthesis and cholesterol reverse transport in the liver and pancreas. Hydrogen sulfide involved in cell protection, inflammation, vascular function, nerve function and mitochondrial function, is generated through areverse sulfur reaction catalyzed by enzymes like cystathione gamma-layse (CTH), cystathione beta-synthase, and 3-mecaptopyruvate sulfur transferase. However, the regulatory mechanism governing CTH expression remains unknown. This study aimed to investigate how LHR-1 controls CTH expression and the impact of hydrogen sulfide on the hepatic accumulation of neutral fat. CTH expression was significantly increased by 24-hour fasting in normal mice. To assess hydrogen sulfide activity, mice were measured for hydrogen sulfide production under non-fasting or 24-hour fasting conditions, and it was confirmed that hydrogen sulfide production was significantly reduced in LRH-1 LKO mice than in WT mice. In conclusion, this study supports the notion that CTH is a target gene for LRH-1 and that LRH-1 deficiency leads to reduced hydrogen sulfide production by downregulating CTH expression. This decrease in hydrogen sulfide production impairs fatty acid oxidation, resulting in the accelerated accumulation of triglycerides in the liver.

Keywords: LRH-1, CTH, hydrogen sulfide, TG accumulation, liver

P6-17

LRH-1 regulates hepatic triglycerides through BHMT in the fasting liver

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LRH-1 is an orphan nuclear receptor expressed in tissues derived from

the endoderm, including the intestines, liver, and exocrine pancreas. It is primarily involved in lipid and cholesterol metabolism, and previous study confirmed that LRH-1 expression was increased in the liver during fasting. Betaine-homocysteine S-methyltransferase (BHMT), one of the most abundant proteins in the liver, is involved in the regulation of homocysteine metabolism. Furthermore, it is known that decreased BHMT gene expression leads to homocysteine accumulation in the liver, which can induce endoplasmic reticulum (ER) stress. In this study, the mechanism of controlling the expression of BHMT by LRH-1 was identified, and the effect of BHMT deficiency on metabolic function was revealed in the liver. During fasting, both BHMT and LRH-1 gene expression increased in the liver of normal mice, but BHMT gene expression was decreased in LRH-1 deficient mice. In addition, the lipid peroxide content in the liver tissues of LRH-1 deficient mice was increased. Promoter activity analysis confirmed the binding of LRH-1 to a specific site at +131 ~ +137 bp of the BHMT promoter. In conclusion, this study suggested that LRH-1-mediated increase in BHMT gene expression alleviates triglyceride accumulation by suppressing reactive oxygen species levels and reducing ER stress.

Keywords: LRH-1, BHMT, fasting liver, triglycerides, homocysteine

P6-18

Imatinib decreased dexamethasone-induced pancreatic β-cell apoptosis via the reduction of GSTP1

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Oxidative stress is one of several mechanisms of Glucocorticiods (GCs)-induced pancreatic β-cell. Our previous study showed that dexamethasone-induced pancreatic β-cell apoptosis by reduction of an antioxidant enzyme, glutathione S-transferase P1 (GSTP1). Previous study suggested that imatinib, a tyrosine kinase inhibitor, had antioxidant effect. Whether imatinib decreases dexamethasone-induced pancreatic β-cell apoptosis via reduction of GSTP1 is still unknown. This study investigated the effect of imatinib to reduce dexamethasone-induced pancreatic β-cell apoptosis through decreased GSTP1. INS-1 cells were treated with 0.1 uM dexamethasone with or without 10 mM imatinib in the presence and absence of RU486. At 48 h, GSTP1, pJNK, Bax (a pro-apoptotic protein) and Bcl_2 (an anti-apoptotic protein) were examined by Western blot analysis. At 72 h, AnnexinV/PI assay was assessed to determine cell apoptosis. Superoxide production was determined by nitroblue tetrazolium (NBT) assay. Dexamethasone significantly increased early cell apoptosis in INS-1 cells when compared to the result of control condition. Imatinib significantly decreased dexamethasone-induced apoptosis of the INS-1 cells. Dexamethasone significantly increased superoxide production when compared to that of the control condition but imatinib with dexamethasone significantly reduced superoxide production. Dexamethasone significantly decreased GSTP1, and Bcl_2 protein expression when compared to those of the control conditions, while pJNK and Bax protein expression were significantly increased by dexamethasone. Imatinib was able to decrease effect of dexamethasone on GSTP1, p JNK, Bax, and Bcl_2 expression. Also, RU846 was able to abolish effect of dexamethasone-decreased GSTP1. Together, our study suggested that imatinib decreases dexamethasone-induced pancreatic β-cell apoptosis through increased GSTP1 expression.

Keywords: Glucocorticoids, Pancreatic β-cell, GSTP1, Apoptosis

P6-19

Long-term testosterone effects on small-conductance Ca^{2+}-activated K^+ currents and expression in human coronary artery endothelial cells

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Background: Testosterone is known to decrease risks for coronary artery disease. Acutely, testosterone causes endothelium-dependent coronary vasodilation via increased NO release. Previously, we had found that testosterone could increase endothelial small-conductance Ca^{2+}-activated K^+ channel (SK_{Ca}) currents, causing hyperpolarization[1] and enhancing NO production[2]. Therefore, we aimed to investigate the effect of long-term testosterone on SK_{Ca} channel current and expression. Both subunits of endothelial SK_{Ca} channel, $K_{Ca}2.2$ and $K_{Ca}2.3$, were examined due to their reported involvement in regulating vascular function and blood flow.
Methods: SK_{Ca} currents and expression in human coronary artery endothelial cells were measured by using whole cell patch clamp technique and western blotting, respectively.
Results: Cultured human coronary artery endothelial cells were incubated for 48 hours in a culture medium containing 30 nM testosterone (physiological concentration). SK_{Ca} current was identified by using a specific SK_{Ca} channel blocker apamin. Compared to control, the apamin-sensitive SK_{Ca} current was significantly increased in testosterone-treated cells (control vs testosterone: 8.07 ± 1.60% (n=7) vs 20.20 ± 3.13% (n=7), $p < 0.05$, unpaired t test). Western blotting showed that membrane protein levels of $K_{Ca}2.2$ and $K_{Ca}2.3$ were significantly increased in testosterone-treated cells while cytosolic protein levels were not changed.
Conclusion: The SK_{Ca} currents and membrane protein expression were increased in the testosterone treated human coronary artery endothelial cells, which could be a mechanism accounting for the long-term beneficial effect of testosterone on endothelium-dependent vascular function.
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Keywords: endothelial cells, small-conductance Ca^{2+}-activated K^+ channel, testosterone

P6-20

Genistein protects against dexamethasone-induced pancreatic β-cell apoptosis through reducing ER stress and txnip expression

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"Steroid-induced diabetes" is a well-known metabolic side effect of prolonged glucocorticoids (GCs) treatment. Endoplasmic reticulum stress (ER stress) has been shown as one of mechanism of GCs-induced pancreatic β-cell apoptosis. Recently studies showed linkage between

ER stress and TXNIP promoting cell apoptosis. However, the mechanism of GCs induced pancreatic β-cell apoptosis via ER stress and TXNIP are still unknown. Genistein is a soy phytoestrogen, protected against toxic agents-induced pancreatic β-cell apoptosis. Therefore, this study aimed to investigate whether genistein protected against dexamethasone-induced pancreatic β-cell apoptosis via reduction ER stress and TXNIP. This study demonstrated that dexamethasone induced pancreatic β-cell apoptosis by cPARP protein expression. Our results showed that dexamethasone increased SERCA, IRE1-α, sXBP pJNK, Bax protein expressions but decreased Bcl-2 protein expression. Furthermore, this study found that dexamethasone up-regulated TXNIP. Co-treatment dexamethasone with genistein decreased cell apoptosis. In addition, genistein reduced SERCA, IRE1-α, sXBP, pJNK and Bax but induced Bcl-2 expressions. Co-treatment dexamethasone with genistein markedly decreased TXNIP. Similar result of cPARP and TXNIP were found in pancreatic islets. Thus, this study demonstrated that genistein protected against dexamethasone-induced pancreatic β-cell apoptosis by decreasing ER stress and TXNIP expression.

Keywords: genistein, dexamethasone, Pancreatic B-Cell apoptosis, ER Stress, TXNIP

Poster

P6-21

Novel biomarkers for diabetic cardiomyopathy FABP3 and IGFB7

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Cardiovascular complications are the main cause of death in diabetes mellitus (DM) patients, Accounting for more than 80% of cases. However, there is no single prognostic test to date that is sensitive enough to detect the early onset of diastolic dysfunction, the first sign of diabetic cardiomyopathy (DCM), while 2D and 3D echocardiograms can detect real-time structural and functional changes in DCM, their biggest limitation is that they cannot detect the initial molecular changes that occur prior to the appearance of structural alteration such as fibrosis and cardiac hypertrophy. In this study, we examined whether FABP3, IGFBP7, and MYL7, may serve as biomarkers, individually or in combination for screening the early onset of the disease.

Keywords: diabetic cardiomyopathy, FABP3, IGFB7, diabetes mellitus

P6-22

Perfluorooctanoic acid suppressed the GABAA receptor-mediated activity in gonadotropin-releasing hormone neurons

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Gonadotropin-releasing hormone (GnRH) neurons are the key regulator of the hypothalamic-pituitary-gonadal (HPG) axis which controls reproduction physiology. GABAergic neurotransmission has long been considered one of the key players in the regulation of GnRH neurons. $GABA_A$ receptors mediate both phasic and tonic effects on GnRH neurons. Various hormones throughout the developmental stage, shape the brain for a time specific physiological events like puberty and reproduction. During the developmental stage exposure to endocrine-disrupting chemicals (EDCs), exert disruptive effects on reproductive development. EDCs are environmentally persistent and findings show that perfluoroalkyl substances (PFAS) are the most toxic EDCs that have adverse effects on reproductive development. Within the past decade, the hazard of PFAS on the HPG axis in mammals has drawn greater attention. To date, no findings are reported about the impact of PFAS on GnRH neuronal regulation and its role in reproductive physiology at the hypothalamic level. Here, we aimed to investigate the effect of perfluorooctanoic acid (PFOA), one of the PFAS on GABA receptor-mediated action in GnRH neurons using the brain slice patch-clamp technique. PFOA suppressed the phasic GABA response in GnRH neurons. In addition, the $GABA_A$ receptor agonist muscimol-mediated response was suppressed by PFOA. The effect of PFOA on GABA response was concentration-dependent and exposure time-dependent. Furthermore, PFOA suppressed the tonic $GABA_A$ receptor agonist THP-mediated responses on GnRH neurons. These findings suggest that PFOA is a potent EDCs that may directly affect reproductive physiology at the hypothalamic level.

Keywords: γ-amino butyric acid, gonadotropin-releasing hormone neurons, Hypothalamic-pituitary-gonadal axis, patch-clamp, perfluorooctanoic acid

P6-23

KiSS-1 can inhibit the invasion of ectopic endometrium in mice endometriosis disease model

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Background: Endometriosis is a common disease in women of childbearing age. Although endometriosis is considered as a benign disease, it exhibits biological behaviour, which is similar to malignant tumours, such as migration and invasion. Over the past decades, even though considerable effort has been devoted to investigate the pathogenesis and aetiology of endometriosis, its pathogenesis is still not fully understood. Kisspeptins, which are a family of neuropeptides encoded by the KiSS-1 gene, were initially identified by Lee et al. in 1996 as suppressors of metastasis in malignant melanoma cells. The initial KiSS-1 gene product consists of a 145-amino acid peptide that is subsequently cleaved to peptides of shorter lengths including kisspeptin-54, -14, -13, and -10, which act through the activation of its receptor, KiSS-1R (G protein-coupled receptor 54, GPR54). Moreover, studies have reported that kisspeptins display anti-metastatic and anti-migration activity in malignant tumours, such as gastric carcinoma, breast cancer and prostate cancer. Therefore, some researchers speculate that KiSS-1 may play a role in endometriosis. Investigation of the potential mechanisms underlying endometriosis is important to provide insights into the currently unknown pathways involved in endometriosis.
Methods: Surgical induction of endometriosis in C57BL/6 female mice were performed in allogeneic mice or via autologous endometrial transplantation. The expression of KiSS-1 was tested immunohistochemically in the model mice via autologous endometrial transplantation. The incidence of endometriosis in the allogeneic model mice was analyzed after Kisspeptins administration. The expressions of VEGF and MMP-9 in the ectopic endometrium and eutopic endometrium in the model mice via autologous endometrial transplantation were determined by immunohistochemical method after Kisspeptins administration.

Results: Kisspeptins are lowly expressed in model mice via autologous endometrial transplantation when compared with control mice. What's more, the administration of Kisspeptins to the allogeneic model mice could reduce the incidence of endometriosis. In the endometriosis model mice via autologous endometrial transplantation, the decreased expression of VEGF and MMP-9 was observed after the administration of Kisspeptins when compared with control group.
Conclusion: The decreased expression of KiSS-1 may be related to the mechanism of endometriosis. KiSS-1 could possibly inhibit the development of endometriosis by suppressing MMP-9 and VEGF expression. These findings may help to understand the pathogenesis of endometriosis.

Keywords: endometriosis, KiSS-1, gene, MMP-9, VEGF

P6-24

The effects of physical exercise and growth hormone on the expression of follicle stimulating hormone receptors in ovarial granulosa cells, and number of ovary follicles of wistar (*rattus norvegicus*) perimenopause

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Perimenopause is characterized by a significant decrease in the functional unit of the ovary, namely the number of follicles. This decrease will result in a decrease in hormone levels originating from the ovaries, resulting in a variety of clinical complaints that can lower the quality of life of women entering menopause. The goal of this study was to show that giving perimenopausal Wistar rats physical exercise and growth hormone (GH) increased the expression of ovarian granulosa cell FSH receptors and the number of ovarian follicles.
This was an experimental study with a randomized post-test only control group design. The study used 24 perimenopausal Wistar rats divided into four groups at the Biomedical Integrated Laboratory, Faculty of Medicine, Udayana University, and the Pathobiology Laboratory, Faculty of Veterinary Medicine, Udayana University. The control group (P0) received aquabidest 0.1 ml subcutaneous injections daily for 30 days, the treatment group 1 (P1) received swimming exercise for 30 minutes 5 times a week for 30 days, the treatment group 2 (P2) received GH injections subcutaneously at a dose of 0.016 IU/0.1 ml daily for 30 days, and the treatment group 3 (P3) received a combination of subcutaneous GH injections at a dose of 0.016 IU/0.1 ml and 30 days of swimming for 30 minutes 5 times a week. FSH receptor expression on ovarian granulosa cells and the number of ovarian follicles were observed and analyzed using One-way ANOVA test at the significance level ($p<0.05$).
The average expression of FSH receptors in the P1 group (62.58±1.11), P2 group (111.67±1.40), and P3 group (132.67±0.82) was significantly higher ($p<0.05$) than in the control group (22.58±1.11). The mean number of primary follicles (10.75±0.52), secondary follicles (7.25±0.52), and tertiary follicles (2.67±0.52) in the P1 group was significantly higher ($p<0.05$) than the control group's average number of primary (2.33±0.52), secondary (1.67±0.41), and tertiary (0.00). The P2 group had a significantly higher number of primary follicles (14.58±0.66), secondary (10.25±0.88), and tertiary (4.33±0.52) than the control group ($p<0.05$) and the mean number of primary (18.33±0.41), secondary (12.25±0.76), and tertiary (6.33±0.61) follicles in the P3 group was significantly higher ($p<0.05$) than in the control group.
According to the findings of the study, physical exercise and GH, as well as their combination, can increase the expression of FSH receptors and the number of follicles in perimenopausal Wistar rats.

Keywords: perimenopause, physical exercise, growth hormone, FSH receptors, ovarian follicles

P6-25

Acute cadmium exposure impairs ion homeostasis of boar spermatozoa *In Vitro*

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Cadmium is a pervasive environmental toxicant known to adversely affect a variety of organs and tissues, leading to complex pathophysiological outcomes. It has detrimental effects on both the male and female reproductive systems, with the male reproductive tract is particularly vulnerable due to vasculature, metabolism, and cadmium-sensitive gene expression. While the significance of cadmium's impact on sperm physiology is established, the effects on sperm ion channels have not been fully elucidated to date. This study aimed to investigate the mechanisms underlying the toxic effects of cadmium on ion channels of mature spermatozoa and its impact on ion homeostasis. The electrophysiological effects of cadmium were assessed using patch-clamping HEK cells overexpressing Slo1, Slo3, and Hv1 ion channels. Changes in intracellular calcium ion concentration, membrane potential, and pH of spermatozoa were measured using time-lapse flow cytometry, while the acrosome reaction was estimated using flow cytometry. Cadmium reduced the maximum current of Slo1 and Hv1 channels in HEK cells while minimally affecting Slo3, consistent with previous findings. Furthermore, cadmium shifted the action potentials of Hv1 to non-physiological voltage ranges, suggesting significant inhibition of the channel by cadmium under normal physiological conditions. In mature boar sperm, cadmium exposure led to dose-dependent reductions in motility parameters, ultimately resulting in immotility. Intracellularly, cadmium increased intracellular calcium levels in an extracellular calcium-independent manner, potentially originating from internal calcium stores. Notably, the membrane potential of boar sperm was not affected by extracellular cadmium exposure, while sperm pH remained relatively unchanged up to a final concentration of 1mM Cd. Additionally, cadmium also increased spontaneous acrosome reaction of sperm. These findings contribute to a better understanding of the detrimental effects of cadmium on the physiology of mature spermatozoa and are crucial for developing targeted interventions to mitigate the harmful effects of cadmium exposure on male reproductive health.

Keywords: cadmium, boar sperm, ion homeostasis, calcium, acrosome reaction

P6-26

Characteristics of breastfeeding mothers with lactational mastitis at the Indonesian Breastfeeding Mothers Association in 2022

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Background: The World Health Organization (WHO) recommends that babies be exclusively breastfed for the first six months to achieve optimal growth, development, and health. However, according to UNICEF data 2018, only 41% of babies under six months were exclusively breastfed. Although most mothers (96%) breastfeed their children in Indonesia, only 40-59% of babies under six months of age are exclusively breastfed. One of the reasons why mothers stop breastfeeding is mastitis. Approximately 3% to 20% of women develop mastitis during lactation, and most episodes occur in the first 6-8 weeks postpartum. This study aims to present a profile of breastfeeding mothers who experience mastitis.
Methods: This cross-sectional descriptive study was conducted January-December 2022 in collaboration with the Indonesian Breastfeeding Mothers Association (Asosiasi Ibu Menyusui Indonesia / AIMI) by distributing research questionnaires. From this study, 334 respondents were collected who filled out a complete questionnaire, with 52 breastfeeding mothers with lactational mastitis as research subjects.
Results: Fifty-two of the 334 respondents (15.6%) had experienced lactational mastitis. Of these 52 subjects, 94.2% are 20-35 years old, 90.4% do not exercise regularly, 76.9% experienced sore nipples, 69.2% do not take supplements containing beta carotene, 53.9% have 4-6 symptoms of depression, 51.9% are exposed to cigarette smoke regularly, 51.9% do not use breast milk booster supplements, 38.5% have 0-1 month old baby, 13.5% have obesity, 11.5% have PCOS, 9 6% had heavy bleeding during childbirth, 3.8% have thyroid hormone disorders, and 1.9% have hypertension.
Conclusion: The majority of breastfeeding mothers with lactational mastitis are between the ages of 20 and 35 and experience sore nipples, frequent exposure to cigarette smoke, and 4-6 depression symptoms. Most do not regularly exercise, take beta-carotene supplements, or use supplements to increase breast milk production.

Keywords: mastitis, breastfeeding, depression symptoms, sore nipples, beta carotene

P6-27

Description of radioactive iodine (RAI) results therapy in patients with well-differentiated thyroid carcinoma (WDTC) at dadi regional hospital and sandi karsa hospital makassar of south sulawesi

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Background: Thyroid Carcinoma or Thyroid Cancer is the most common endocrine malignancy. Approximately 3.4% incidence of thyroid cancer of all cancers diagnosed each year. Thyroid carcinoma is classified into 2 parts based on its histology, namely differentiated and undifferentiated thyroid carcinoma. In Nuclear Medicine there is the term theranostics which is a combination of diagnostic tools that help to carry out therapy as well as appropriate diagnostic steps for a disease. One of the specific steps in the management of well-differentiated thyroid carcinoma is Radioactive Iodine (RAI) which has been the key to the treatment of well-differentiated thyroid carcinoma for decades.
Research Objectives: To find out how the results of Radioactive Iodine (RAI) therapy in well-differentiated thyroid carcinoma patients in the working area at Dadi Regional Hospital and Sandi Karsa Hospital Makassar of South Sulawesi.
Methods: The design of this study was carried out using a descriptive method and a retrospective approach which aims to see the proportion distribution of the results of therapy for patients with well-differentiated thyroid carcinoma in the working area at Dadi Regional Hospital and Sandi Karsa Hospital Makassar of South Sulawesi.
Results: The results of this study showed that in the initial RAI therapy results, 60.6% of WDTC patients found no regional lymph node metastases or distant metastases, 24.2% regional lymph node metastases were detected, 12.1% bone disease metastases, and 3.0% metastases deep in the lung fields.
Conclusion: Appearance and absence of metastases in patients with well-differentiated thyroid carcinoma (WDTC) can be reviewed through ablation therapy I-131 Radioactive Iodine Therapy.

Keywords: WDTC, RAI, Ablation, I-131

P6-28

Protective effects and mechanism of 17β- estradiol and progesterone on cardiomyocytes damaged by high glucose and insulin resistance

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Diabetic cardiomyopathy (DCM), a major complication of diabetes, is characterized by cardiac hypertrophy, ventricular structural changes, and dysfunction of diastolic and systolic, which increases the risk of heart failure in diabetics. Hyperglycemia and hyperinsulinemia are the most important causes of DCM pathogenesis. The incidence of DCM is increased significantly in postmenopausal women and in men compared with premenopausal women, suggesting that 17β-estradiol and progesterone have beneficial effects on the occurrence and develop-

ment of DCM. Therefore, in the present study, H9c2 cardiomyocytes were treated with hyperglycemia and hyperinsulin respectively to establish cell hyperglycemia injury and insulin resistance models, and to investigate the intervention effects and mechanisms of 17β-estradiol and progesterone on H9c2 cardiomyocytes subjected to hyperglycemia and hyperinsulinism, which aimed to provide the theoretical basis for the treatment of diabetic cardiomyopathy. In the present study, it was found that 250mM glucose and 1× 10-7M insulin could successfully induce a model of H9c2 myocardial cell high glucose injury and an insulin resistance model respectively, 17β-estradiol and progesterone inhibited the expression of pro-apoptotic genes Bax mRNA and protein and also reduced Cleaved caspase-3 and caspase-3 levels, but increased the expression of Bcl-2. At the same time, lactate dehydrogenase, an enzyme linked to cell damage and heart disease, was reduced in H9c2 cells after being treated with both hormones. They could also increase the expressions of PI3K, Akt, AMPK, GLUT-4, and their phosphorylation levels. The results suggest that estradiol and progesterone can inhibit the apoptosis of cardiomyocytes induced by high glucose and accelerate the uptake of glucose through the PI3K/Akt and AMPK pathways, thereby delaying and preventing the occurrence of diabetic cardiomyopathy.

Keywords: H9c2 cardiomyocytes, 17β-estradiol, progesterone, apoptosis, insulin resistance

P6-29

Association of mucin-1 with peripheral diabetic neuropathy in Type 2 Diabetes

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Background: Diabetic peripheral neuropathy (DPN) is one of the most debilitating complications of diabetes. DPN is a major cause of foot ulceration and neuropathic pain. Early detection of DPN is important to reduce morbidity and mortality. There is a need to develop non-invasive and accurate assessment markers. Therefore, we aimed to identify cytokines as novel biomarkers of DPN in type 2 diabetes (T2DM).
Methods: The first stage of the study included 33 subjects (20 diabetic patients without diabetic complication, 13 diabetic patients with only DPN, and 10 subjects with normoglycemia) for cytokine microarray analysis. Blood samples of the subjects were assessed for 310 cytokines to identify potential indicators of DPN. The second stage included 294 subjects (125 diabetic patients without diabetic complication, 69 diabetic patients with only DPN, and 100 subjects with normoglycemia) to validate the potential cytokine associated with DPN. We measured expression of mucin-1 in sciatic nerves of db/db mice using Western blot analysis.
Results: We identified 13 cytokines that differed by 1.5-fold or more in at least one out of the three comparisons (normoglycemia vs. T2DM, normoglycemia vs. DPN, and T2DM vs. DPN) among 310 cytokines. Finally, we selected mucin-1 and validated this finding to determine its association with DPN. Plasma mucin-1 levels were found to be increased in T2DM patients with DPN compared to those in T2DM patients without DPN or subjects with normoglycemia. In db/db mice, plasma mucin-1 level was increased while the expression level of mucin-1 in sciatic nerve was decreased.
Conclusion: Plasma mucin-1 levels were associated with DPN in T2DM. We suggest that mucin-1 may be a promising biomarker for the early detection of DPN. Further studies are needed to evaluate the relationship and mechanism between mucin-1 and DPN in T2DM.

Keywords: type 2 Diabetes, peripheral diabetic neuropathy, mucin-1

P6-30

Role of protein Z in Type 2 Diabetes

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Background: Type 2 diabetes mellitus (T2DM) is a progressive metabolic disease. Early detection of prediabetes is important to reduce the risk of T2DM. Protein z is a member of the coagulation cascade, but its role in diabetes is unknown. Therefore, we aimed to identify protein z as a novel biomarker of T2DM and to elucidate its role in T2DM.
Methods: The first stage of the study included 43 subjects (13 subjects with newly diagnosed T2DM, 13 with prediabetes, and 16 with normoglycemia) for cytokine microarray analysis. Blood samples of the subjects were assessed for 310 cytokines to identify potential indicators of prediabetes. The second stage included 142 subjects (36 subjects with T2DM, 35 with prediabetes, and 71 with normoglycemia) to validate the potential cytokines associated with prediabetes. We measured the hepatic expression of protein z in high fat diet (HFD) or db/db mice using Western blot analysis.
Results: We identified 41 cytokines that differed by 1.5-fold or more in at least one out of the three comparisons (normoglycemia vs. prediabetes, normoglycemia vs. T2DM, and prediabetes vs. T2DM) among 310 cytokines. Finally, we selected protein z and validated this finding to determine its association with prediabetes. Plasma protein z levels were found to be decreased in patients with prediabetes (1,490.32±367.19 pg/mL) and T2DM (1,583.34±465.43 pg/mL) compared to those in subjects with normoglycemia (1,864.07±450.83 pg/mL) ($P<0.001$). There were significantly negative correlations between protein z and fasting plasma glucose ($P=0.001$) and hemoglobin A1c ($P=0.010$). In addition, liver protein z levels were reduced in 20-week HFD or 6-week-old db/db mice.
Conclusion: Plasma protein z levels were associated with prediabetes and T2DM. We suggest that protein z may be a promising biomarker for the early detection of prediabetes. The reduced heptic protein z might contribute to the development of coagulation disorders and vasculopathy in T2DM. Further studies are needed to evaluate the relationship and mechanism between protein z and T2DM.

Keywords: type 2 Diabetes, protein z

P6-31

The event of cataract incidence in patients with history of diabetes mellitus at tarakan hospital, central Jakarta in 2020

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Background: Diabetes mellitus (DM) is a chronic disease in which blood glucose levels increase due to insufficient insulin production in the pancreas or insulin cannot be used effectively by the body (IDF, 2019). Diabetes mellitus is a systemic disease that most often causes

complications of cataracts. Cataracts caused by diabetes mellitus are called diabetic cataracts. In one study using Riskesdas data in 2007, it was found that 18% of the 1,565 patients with diabetes mellitus suffered from cataract complications.
Objective: This study aims to describe the incidence of cataracts in patients with a history of diabetes mellitus at Tarakan Hospital in 2020.
Methods: This study is a descriptive study with quantitative data collection and cross-sectional design. The data source in this study was secondary data from cataract patients who had a history of diabetes mellitus in 2020 at Tarakan Hospital, Central Jakarta.
Results: The results of the study found that there were 260 cataract patients, but those with a history of diabetes mellitus were (25%). Cataract patients who had a history of diabetes mellitus mostly occurred in women as many as 39 patients from 133 patients, while men as many as 26 patients from 127 patients and the majority occurred in the 49-63 year age group with 42 patients.
Conclusion: Patients who have diabetes mellitus have the possibility of cataract complications, especially in the female sex and in the 49-63 year age group.

Keywords: cataract, central jakarta, history of diabetes mellitus, tarakan hospital

Poster

P6-32

A combination of dapagliflozin and metformin ameliorates diabetic nephropathy by suppressing oxidative stress, inflammation, apoptosis, and activating autophagy in diabetic rats

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Early detection and clinical intervention are of major importance in the prevention of diabetic nephropathy (DN) progression. Following consideration of the complementary mechanisms of sodium glucose cotransporter (SGLT2) inhibitors and metformin on the kidney, it is postulated that a combination of both agents could afford greater protection against DN. In this study, a type 2 diabetic rat model induced by high-fat diet/low dose streptozotocin was used to examine the potential protective effects of dapagliflozin, metformin, and a combination of the two on kidney injury with investigation into their cellular signaling mechanisms. The diabetic (DM) rats were given dapagliflozin (1.0 mg/kg/day) or metformin (100 mg/kg/day) or the combined treatment (dapagliflozin 0.5 mg/kg/day plus metformin 50 mg/kg/day) by oral gavage for 4 weeks. In DM rats, dapagliflozin as a monotherapy or in combination with metformin was more effective than metformin alone in attenuating renal dysfunction, improving renal organic anion transporter 3 expression, and activating renal autophagy via modulation of the AMPK/mTOR/SIRT1 axis. Interestingly, dapagliflozin 1.0 mg/kg/day had greater efficacy in suppressing renal oxidative stress in DM rats than metformin or the combined treatment. The renal apoptosis and histopathology of renal and pancreatic tissues in DM rats were improved by both the dapagliflozin monotherapy and the combined treatment. Thus, it appeared that low dose combination treatment with dapagliflozin plus metformin, through synergistic coordination, could modulate oxidative, autophagic, and apoptotic signaling, and confer significant renoprotective effects against DM-induced complications and tissue injury. In addition, a low dose of the combined drug treatment might be beneficial in patients in order to avoid the risk of side effects of medication in diabetes. Future clinical trials are necessary to study the nephroprotective effect of the combined therapy between dapagliflozin and metformin at low dosage in patients with diabetes.

Keywords: diabetic nephropathy, dapagliflozin, metformin, Oat3 function, pancreatic injury

P6-33

Hypothalamic FGF1 regulates systemic glucose and energy homeostasis

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The central nervous system (CNS) plays a key role in the complex interplay of organ communication networks that regulate systemic glucose and energy homeostasis. CNS signaling pathways have been widely investigated as potential targets for the treatment of diabetes and obesity. Recent studies showed that intracerebroventricular administration of recombinant Fibroblast Growth Factor 1 (FGF1) elicited potent and sustained glucose normalization in hyperglycemic rodent models; however, the function of endogenous brain FGF1 in the context of metabolic regulation remains unknown. FGF1 null mice, on the other hand, have previously been shown to develop severe insulin resistance when fed a high-fat diet. However, a limitation of these studies is that it is unclear which tissue/organ mediates insulin resistance caused by whole-body FGF1 depletion. To investigate the *in vivo* function of brain FGF1, we generated mice with a neuron-specific deletion of FGF1 (FGF1 BKO) and observed that these mutant mice have impaired glucose tolerance. FGF1 BKO mice had decreased ERK/STAT3 signaling in the ventromedial hypothalamus (VMH) and arcuate nucleus (ARC), suggesting reduced leptin sensitivity, and reduced AMPK signaling in muscle which may contribute to dysregulated glucose control. Analysis of endogenous brain FGF1 expression revealed that FGF1 expression increased in specific regions of the hypothalamus including the VMH upon glucose administration or refeeding conditions. Interestingly, AAV-Cre-mediated deletion of FGF1 in the adult VMH led to body weight gain in addition to glucose intolerance. VMH-specific deletion of FGF1 also resulted in increased adiposity. Taken together, we propose that hypothalamic FGF1 regulates glucose and energy homeostasis through modulating VMH leptin sensitivity.

Keywords: ventromedial hypothalamus, energy homeostasis, glucose homeostasis, fgf1, leptin

P6-34

Liver coxsackievirus and adenovirus receptor disruption develop nonalcoholic fatty liver diseases

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Background: Coxsackievirus and adenovirus receptor (CAR) is expressed in vascular endothelial cells and hepatocytes, localizing at the tight junction complexes. However, the primary playing role of CAR in the hepatocyte cell-to-cell junction and lipid transport was not proven

yet. CAR is an essential regulator of epithelial tight junction homeostasis and endothelial paracellular permeability. Hepatocyte CAR disruption may induce nonalcoholic fatty liver diseases (NAFLD).
Methods: CAR expression was observed from human Nonalcoholic steatohepatitis (NASH) patient liver tissue using immunohistochemistry. To define the molecular function of CAR in the liver, we generated liver-specific CAR knockout mice using a CAR floxed allele with Albumin Cre mice. Mice were fed a calorie 60% fat diet for 16 weeks. Body weight change and liver lipid accumulation were observed. The novel interaction molecule was defined by pull-down assay and mass-spec.
Results: CAR expression was dramatically reduced from fatty liver disease patient liver tissue. Liver-CAR deletion induced lipid accumulation in the hepatocyte at 8-month-old ages. In 16 weeks high-fat diet, CAR knockout mice developed non-alcoholic fatty liver diseases and increased blood glucose levels with inflammatory cell infiltration in the liver. CAR-deleted hepatocytes showed the induction of ApoB and LDLR protein expression and lipid transport.
Conclusion: CAR disruption may affect the regulation of lipid transport and storage in hepatocytes. CAR plays an essential role in lipid accumulation in the liver and may be a novel therapeutic target for NAFLD.

Keywords: coxsackievirus and adenovirus receptor, fatty liver, nonalcoholic steatohepatitis, NAFLD

P6-35

Neddylation attains bone homeostasis by regulating osteoclastogenesis and osteoblastogenesis

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Background: The regulation of osteoblast and osteoclast is critical to maintain physiological bone homeostasis and prevent bone-destructive diseases like osteoporosis. Many studies have extensively investigaed the post translational modifcaiton(PTMs) landscape of the nuclear factor of activated T-cells calcineurin-dependent 1(NFATc1), an essential role in osteoclastogensis, and Runt-related transcription factor 2(RUNX2), the first transcription factor required for osteoblastogenesis. However, the role of neddylation, a vital post-translational modification regulated by the NEDD8 conjugation pathway, remains unexplored. Recently, neddylation has been shedding lights on numerous diseases as a therapeutic target. Despite the recognition of role in neddylation in various diseases such as obesity and neurodegeneration, its role in bone diseases is yet unperceived.
Methods: Mouse bone marrow macrophages (BMMs) were subjected to stimulation with macrophage colony-stimulating factor (M-CSF) and receptor activator of NF-Kb ligand (RANKL). Simultaneously, primary osteoblast isolated from neonatal mouse clavaria were cultured with BMP2, ascorbic acid, and beta glycerophosphate. Western blot, immunoprecipiation, luciferase reporter assay, and RT-qPCR were conducted to examine the function of neddylation. To assess the effect of the neddylation inhibitor in an osteoporosis mouse model, we performed ovariectomy(OVX) on 8-week-old mice, followed by a 4-week treatment with the neddylation inhibitor. Subsequently, bone mineral density(BMD) of femur was measured using micro-CT imaging.
Results: Here, we demonstrated that neddylation inhibition bidirectionally regulated bone formation controlling both osteoclastogenesis and osteogenesis. The process of neddylation enhanced the transcriptional activity and protein stability of NFATc1 by inhibiting ubiquitin-mediated proteasomal degradation. However, neddylation of Runx2 was found to enhance ubiquitin-mediated proteasomal degradation in osteoblasts, revealing a reciprocal role in maintaining bone homeostasis. As expected, Neddylation inhibitors were observed to impede osteoclast differentiation and promote osteoblast differentiation during the process of differentiating osteoblasts and osteoclasts. In order to verify the efficacy of neddylation inhibitors in osteoporotic disease, we administered these inhibitors to ovariectomized mice. Consequently, the observed improvements in bone mineral density (BMD) indicators, including BV/TV (bone volume fraction) and trabecular thickness, provided confirmation of the positive effects. These results further supported the potential of neddylation inhibitors in the treatment of osteoporosis.
Conclusion: This study indicates that neddylation may contribute in accomplishing bone homeostasis and providing a novel therapeutic strategy for osteoporosis treatment.

Jooseung Lee and Min Young Lee equally contributed in this research.

Keywords: neddylation, NFATc1, RUNX2, osteoclastogenesis, osteoblastogenesis

P6-36

Effects of cannabidiol on primary bone cells from skeletally mature ovariectomized rats

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Cannabidiol (CBD) as a cannabinoid receptor agonist is a primary non-psychotic compound found in cannabis with several positive actions including bone health. This study examined the effect of CBD on primary osteoclasts and osteoblasts isolated from skeletally mature Sprague-Dawley rats. At the age of 26 weeks old, nulliparous rats were undergone either sham operated (SHM) or ovariectomized (OVX). Two weeks after the operation, bone marrow was isolated from femurs and tibiae and cultured in α-MEM supplemented with 10 ng/mL M-CSF in 96-well plates at 2×10^4 cells/well to differentiate into osteoclasts. After 24 h, the medium was changed to osteoclast differentiation medium containing 10 ng/mL of M-CSF and RANKL. The osteoclast cultures were fixed with 4% paraformaldehyde and stained for tartrate-resistant acid phosphatase (TRAP). Osteoblasts were derived from explant cultures of femurs and tibiae in DMEM supplemented with L-ascorbic acid 2-phosphate. When the cultures reached confluence, the culture were passaged and plated in 96-well at 1×10^4 cells/well, respectively. Osteoblasts in 96-well plates were allowed to settle overnight and CBD at 0, 0.1, 0.5, 1, 5, 10, and 20 μM was added to the culture. After 48-h incubation, MTT assay was performed to evaluate cell viability. Here, we observed that CBD at the concentrations of 1, 5, and 10 μM suppressed osteoclast multinucleation in a dose-dependent manner. Specifically, 1 μM CBD significantly reduced the number of multi-nucleated cells (MNCs) when compared to the control, while 10 μM CBD almost completely suppressed osteoclast differentiation. These results were consistent in both osteoclasts isolated from SHM and OVX rats. For osteoblast culture, CBD at the concentration of 0.1–1 μM did not affect the growth of osteoblasts, whereas higher doses (5, 10 and 20 μM) increased pre-osteoblast proliferation as compared to the controls. Our findings demonstrated that CBD at 5 μM or higher presented favorable effect towards increasing osteoblast cell viability, whereas CBD at the dose beginning from 1 μM inhibited

MNC formation. In conclusion, CBD was able to regulate osteoclast and osteoblast functions; therefore, it could be used to modulate bone turnover towards bone formation.

Keywords: cannabidiol, CB2 agonist, osteoblasts, osteoclasts, bone cells

P6-37

The association between body physiologic parameters and rate pressure product (RPP) in young adults with obesity and overweight

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Background: A real solution is needed for the global health issue of overweight and obesity. Over 1.9 billion persons over 18 were overweight in 2016, and over 650 million were obese. If current trends continue, more than 4 billion people, or 51% of the world's population, will be overweight or obese by 2035. Obese or overweight people are far more likely to develop degenerative illnesses, including hypertension, diabetes mellitus, and other conditions of the cardiovascular and metabolic systems. The RPP (Rate Pressure Product) assesses myocardial oxygen demand and cardiac workload as an early indicator of the risk of developing cardiovascular and metabolic diseases.
Aims: The purpose of this study was to assess the association of several physiological parameters such as body weight, height, systolic blood pressure, diastolic blood pressure, heart rate, and body mass index (BMI) with RPP in young adults with overweight and obesity.
Methods: A cross-sectional study was conducted on 62 overweight and obese young adults who met the inclusion and exclusion criteria. The subject was measured for physiological parameters such as body weight, height, systolic blood pressure, diastolic blood pressure, heart rate, and BMI. RPP was assessed by systolic blood pressure (SBP) and heart rate (HR). Statistical analysis was done using multiple linear regression tests.
Results: Based on the results of multiple linear tests, it was found that 99.7% of the independent variable simultaneously affected the dependent variable (R square 0.997), and body weight, height, systolic blood pressure, and heart rate, BMI significantly affected RPP ($p < 0.05$).
Conclusion: Several physiological parameters (body weight, height, systolic blood pressure, heart rate, and BMI simultaneously have a significant effect on RPP in young adults with overweight and obesity. Because RPP is an early predictor of cardiovascular disease risk, early prevention can be undertaken to prevent the disease burden at overweight-obese young adults by modulating several physiological parameters.

Keywords: body physiological parameters, rate pressure product, RPP, overweight, obesity

P6-38

Effect of lunar phases on neutrophil-to-lymphocyte ratio in type-2 diabetic subjects

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Background: Lunar phases influence fasting plasma glucose (FPG), oxidative stress, inflammatory status, and foot temperature (FT) of type 2 diabetic (T2DM) subjects. There is an evidence of the association of elevated foot temperature (FT) and neutrophil-to-lymphocyte ratio (NLR), a marker of inflammation, with the pathogenesis of diabetic peripheral neuropathy (DPN) in T2DM individuals. The present study was aimed to evaluate the changes of some hematological parameters with special reference to the changes of NLR in T2DM subjects and to assess the association between NLR and FPG, oxidative stress markers like malondialdehyde (MDA) and glucose 6 phosphate dehydrogenase (G6PDH), other inflammatory marker like tumor necrosis factor alpha (TNF-α) and FT in T2DM subjects at different lunar phases such as third quarter (TQ), new moon (NM), first quarter (FQ) and full moon (FM).
Methods: This cross-sectional study was conducted on 88 (44 males and 40 females) randomly selected T2DM subjects at different lunar phases. The fasting plasma glucose and haemoglobin concentration (gm%) of each subject in each lunar phase were measured. Total count (TC) and differential count (DC) of white blood cells (WBC) were measured using an automated hematology analyzer. Dorsal and plantar surface temperatures of the feet were measured by infrared dermal thermometer. Oxidative stress markers like MDA and G6PDH were measured by the spectrophotometric method. Inflammatory marker like TNF-α was also assessed by *enzyme-linked immunosorbent assay* (ELISA) method.
Results: Hemoglobin concentration ($p<0.01$), neutrophil ($p<0.05$) and NLR ($p<0.01$) were significantly higher in NM and FM compared to that of TQ and FQ. FT, MDA and TNF-α were significantly increased ($p<0.01$) and G6PDH was significantly decreased ($p<0.05$) at the new moon (NM) and full moon (FM) compared to that of third quarter (TQ) and first quarter (FQ) phases of the lunar cycle. There was a significant ($p<0.05$) correlations between NLR and mean FT, FPG, MDA, G6PDH, and TNF-α at NM and FM days in study subjects.
Conclusion: The present study indicated that lunar phases may affect NLR as well as the association between NLR and FPG, MDA, G6PDH, TNF-α and FT in aged T2DM individuals, which might be due to the existence of the biological rhythm interaction with lunar electromagnetic radiations.

Keywords: fasting plasma glucose, lunar phase, neutrophil-to-lymphocyte ratio, oxidative stress, foot temperature

Poster

P7-1

Transcription factor ZFHX3 arrests cell cycle inducing oligodendrocyte differentiation

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Background: Oligodendrocytes (OLGs) are generated from oligodendrocyte precursor cells (OPCs) that exit the cell cycle and differentiate into OLGs throughout the central nervous system at predictable developmental ages. However, the signal transduction mediators underlying these events remain poorly understood. We have previously demonstrated that the transcription factor ZFHX3 (also known as ATBF1) induces cell cycle arrest associated with neuronal differentiation; however, its role in OLG development remains unknown. In this study, we investigated the role of ZFHX3 in OPC cell cycle arrest and its impact on the development of OLGs.
Methods: OPCs were prepared from mixed glial cells from postnatal day 1 (P1) Wistar rats and then differentiated into OLGs with Neurobasal A + 10 ng/ml CNTF. The expression levels of ZFHX3, markers specific to both OPCs and OLGs, and cell cycle-related genes such as p21, p57, and inhibitor of differentiation 4 (Id4) were assessed using various techniques, including real-time PCR, Western blotting, and immunofluorescence staining. Cell proliferation and cell death were determined using BrdU staining and TUNEL assays, respectively.
Results: ZFHX3 was predominantly expressed in the cytoplasm of proliferating OPCs. In contrast, it was primarily localized in the nuclei of differentiated OLGs, where ZFHX3 expression was higher than that in OPCs. ZFHX3 knockdown in OPCs by siRNA resulted in an increase in the number of BrdU- and PDGFR-α-positive cells, along with increased levels of PDGFR-α mRNA and protein. Nevertheless, ZFHX3 knockdown led to a decrease in the expression levels of myelin-related markers, including CNPase and MBP, and reduced the outgrowth of myelin membrane sheets, without affecting cell death. ZFHX3 knockdown in OPCs also decreased the CDK inhibitors (p21 and p57) while increasing Id4 levels at both mRNA and protein levels.
Conclusion: We propose that nuclear ZFHX3 plays an important role in OLG differentiation and maturation by arresting the OPC cell cycle.

Keywords: ZFHX3, cell cycle, oligodendrocyte differentiation

P7-2

Sustained activation of canonical transient receptor potential ion channels in excitotoxicity brain injury models

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Our study examines the postulate that the highly Ca^{2+} permeable TRPC channels ubiquitously coupled to $G_{\alpha q}$ - type G Protein – coupled receptors (GPCRs), notably the group I metabotropic glutamate receptors (mGluR), are a significant driver of excitotoxic brain injury. Following neonatal delivery of the AAVrh20-pCAG-GCaMP5g genetically-encoded Ca^{2+} reporter, in adult mouse, bath application of glutamate (4 mM), or the group I mGluR agonist (S)-3,5-dihydroxyphenylglycine (100 µM), produced significantly less Ca^{2+} loading of Purkinje cell neurites in *Trpc3* null cerebellar brain slices compared with wildtype (WT). Purkinje neuron dendritic (molecular) layer necrosis with long-term (60 minute) exposure to glutamate (1 mM) in cerebellar brain slices from GAD67-GFP knock-in reporter mice was significantly greater than that imaged in slices from *Trpc3* null - GAD67-GFP mice. The contribution of the TRPC - mediated pathophysiology to secondary brain injury expansion *in vivo* was evaluated utilising *Trpc3 null* and *Trpc1,3,6,7* quad knockout (QKO) mouse lines in a photothrombotic focal ischemia infarct model with dual targeting of cerebellar and cerebral cortex regions. Compared with the substantial secondary brain injury expansion over four days post-injury in WT mice, significant neuroprotection was conferred by the absence of TRPC channel expression, with greater injury prevention afforded by the *Trpc1,3,6,7* QKO model. Overall, this study establishes that TRPC channels coupled to $G_{\alpha q}$ - type GPCRs are prominent contributors to the pathophysiological drive for brain injury expansion, generating broad vulnerability of neurons and glia to the release of the full suite of excitatory neurochemicals following brain injury. These findings contribute to the knowledge-base needed to forge a translational pathway addressing the lack of treatment options for stroke, traumatic brain injury, and other degenerative brain disorders associated with neurotransmitter dysregulation.

Keywords: Trpc knockout mice, photothrombotic stroke model, focal ischemia, Gαq - type G Protein – coupled receptors (GPCR), GCaMP Ca^{2+} reporter

P7-3

WITHDRAWN

P7-4

VPS13B regulates morphology of mitochondria and mitophagy

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The VPS13B protein (Vacuolar Protein Sorting 13 Homolog B) is a large membrane-transmembrane protein associated with a rare genetic disease called Cohen syndrome and is characterized by intellectual disabilities, facial deformities, or obesity. Although cellular functions of VPS13B in Trans-Golgi network were described, the roles of VPS13B on other cellular organelles are not fully understood. In this study, we found that loss of VPS13B caused abnormalities in mitochondrial quality control using VPS13B KO HeLa cell lines. Our electron microscopy and immunostaining analysis showed that VPS13B KO cells have enlarged mitochondria compared to WT. In addition, in VPS13B KO HeLa cells, the membrane potential, and dynamics of mitochondria were significantly reduced compared to WT raising its possible role on mitochondria. To

further investigate its cellular function on regulation of mitochondria, transcriptomic analysis was performed. Our transcriptomic analysis showed that mitochondria associated genes or autophagy-related genes are dysregulated. Interestingly, mRNA expression level of DCN, a mitophagy associated proteoglycan, was significantly reduced supporting cellular defect on the fission of mitochondria or dysregulation of mitochondria. Indeed, mitochondrial proteins such as TIM23, ubiquitinated parkin or PINK1 accumulated in VPS13B KO cells. Moreover, mitophagy flux was impaired leading to accumulation of damaged mitochondria in VPS13B KO cells. Taken together, we provide novel roles of VPS13B on the fission of mitochondria and mitophagy.

Keywords: Cohen syndrome, mitophagy, mitochondria, autophagy

P7-5

Increased intrinsic excitability of neurons in the prefrontal cortex by prenatal exposure to high cortisol downregulating dopaminergic and PKA-mediated signaling cascades in rats

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We previously reported that rat pups (Corti.Pups) born from rat mothers that were repetitively injected with corticosterone (s.c., 20 mg/kg/day, 21 days) during pregnancy, exhibited ADHD-like behaviors and delayed cognitive functions. In this study, we investigated the cellular mechanisms underlying ADHD-like phenotypes observed in Corti.Pups, targeting the neurodevelopmental impairments of their prefrontal cortex (PFC). In results, we confirmed that using the enzyme-linked immunosorbent assay, both BDNF and cAMP levels were significantly reduced in the PFC of Corti.Pups, compared to that of the control group (Nor. Pups). mTOR and PKA, which are dominant factors for neurodevelopmental signaling, were also less expressed in Corti.Pups. This signaling downregulation clearly affected the neuronal development, showing lower expression of PSD-95 in PFC of Corti.Pups. Furthermore, in electrophysiological studies, cortical neurons of Corti.Pups exhibited higher excitability of plasma membrane in supra- and subthreshold ranges. This BDNF-mediated downregulation of neurodevelopmental signaling observed in Corti.Pups, seemed to be attributed to the dopaminergic dysregulation in the PFC, because these pups showed the lower level of dopamine and higher expression of dopamine D1 receptor. Our results clearly provide evidence that prenatal exposure to high cortisol disrupts the neurodevelopment of PFC neurons via downregulating dopaminergic and PKA-mediated signaling cascades, possibly triggering the pathogenesis of neuropsychiatric disorders such as ADHD (Korean Government Research Fund, Grant No. NRF-2022R1I1A3063177).

Keywords: ADHD, prefrontal cortex, dopamine, PKA, BDNF

P7-6

KCC2 downregulation after sciatic nerve injury enhances motor function recovery

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Injury to mature neurons induces downregulated KCC2 expression and activity, resulting in elevated intracellular [Cl^-] and depolarized GABAergic signaling. This phenotype mirrors immature neurons wherein GABA-evoked depolarizations facilitate neuronal circuit maturation. Thus, injury-induced KCC2 downregulation is broadly speculated to similarly facilitate neuronal circuit repair. We test this hypothesis in spinal cord motoneurons injured by sciatic nerve crush, using transgenic (CaMKII-KCC2) mice wherein conditional CaMKIIα promoter-KCC2 expression coupling selectively prevents injury-induced KCC2 downregulation. We demonstrate, via an accelerating rotarod assay, impaired motor function recovery in CaMKII-KCC2 mice relative to wild-type mice. Across both cohorts, we observe similar motoneuron survival and re-innervation rates, but differing post-injury reorganization patterns of synaptic input to motoneuron somas – for wild-type, both VGLUT1-positive (excitatory) and GAD67-positive (inhibitory) terminal counts decrease; for CaMKII-KCC2, only VGLUT1-positive terminal counts decrease. Finally, we recapitulate the impaired motor function recovery of CaMKII-KCC2 mice in wild-type mice via local spinal cord injections of bicuculline ($GABA_A$ receptor blockade) or bumetanide (lowers intracellular [Cl^-] by NKCC1 blockade) during the early post-injury period. Thus, our results provide direct evidence that injury-induced KCC2 downregulation enhances motor function recovery and suggest an underlying mechanism of depolarizing GABAergic signaling driving adaptive neuronal circuit reconfiguration that preserves appropriate excitation-inhibition balance.

Keywords: KCC2, motoneuron, motor recovery, spinal cord

P7-7

Macrophage-derived cathepsin S promotes axon regeneration via fibroblasts after peripheral nerve injury

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The peripheral nervous system, unlike the central nervous system, has the ability to regenerate after injury. Recent studies have reported that cross-talk between macrophages and Schwann cells (SCs) at the injured site of peripheral nerves is involved in axon regeneration; however, the underlying mechanisms are not fully understood. Here, we show the quadripartite signaling relay of macrophage–fibroblast–SC–axon during axon regeneration. Transcriptome analysis revealed that cathepsin S (Ctss) mRNA, a macrophage-selective lysosomal protease, was upregulated in the injured inferior alveolar nerve (IAN) compared to the intact IAN in Sprague-Dawley rats. CTSS immunoreactivity was restricted in macrophages at the injured site. Hypoesthesia in the mandibular region after IAN transection (IANX) was partially recovered from 12 days after injury, which was abrogated by macrophage ablation at the injured site.

Additionally, a robust induction of c-Jun, a marker of the repair-supportive phenotype of SCs, after IANX was abolished by macrophage ablation. Behavioral analysis showed that local administration of CTSS at the injured site promoted sensory recovery from hypoesthesia, whereas pharmacological inhibition or genetic silencing of CTSS at the injured site inhibited sensory recovery. Adoptive transfer of M2-polarized macrophages at the site facilitated macrophage CTSS-dependent sensory recovery. Post-IANX, CTSS cleaved Ephrin-B2 in fibroblasts, which in turn bound to EphB2 on SC. CTSS-induced Ephrin-B2 cleavage was also observed in human sensory nerves. The facilitation of sensory recovery by CTSS was inhibited by the administration of Ephrin-B2 blocking peptide at the injured site, which also inhibited c-Jun induction. We also confirmed that CTSS initiated Ephrin-B2-mediated fibroblast-SC signaling in primary culture fibroblasts and SCs from the trigeminal nerves. CTSS-induced increase in c-Jun-positive SCs in co-culture was markedly suppressed by CTSS inhibitor or Ephrin-B2 blocking peptide. CTSS did not directly influence axon outgrowth in primary TG neurons. These results suggest that macrophage-derived CTSS contributes to axon regeneration by activating SCs via Ephrin-B2 shedding from fibroblasts.

Keywords: cathepsin s, macrophage, regeneration, peripheral nerve, hypoesthesia

P7-8

Developmental up-regulation of voltage-gated Na^+ channel and its electrophysiological function in rat hippocampal neurons

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As hippocampal neurons mature during neonatal development, they pass through plenty of changes such as morphology, synaptic pruning, and electrical activity. In this study, we report evidences for the developmental enhancement of electrical activity in rat hippocampus. To observe the electrophysiological property of hippocampal neurons in stages from DIV 5 to DIV 20, we measured diverse parameters of action potential (AP) including resting, maximum, minimum potential, AP height, and max potential latency. We also measured voltage-gated Na^+ currents via inverse [Na^+] gradient voltage-clamp. The results show that DIV 20 hippocampal neurons generated 40 mV higher maximum potential and ~2-fold larger AP height than DIV 5 neonatal neurons. In addition, DIV 20 hippocampal neurons reached to max potential within ~1 ms after suprathreshold current injection. It is ~4 fold faster than DIV 5 max potential latency. Also, voltage-gated outward Na^+ current density and window current probability was gradually increased during the identical stages. To identify the subtypes of voltage-gated Na^+ channels (VGSCs) up-regulated, we examined mRNA expression levels of all 9 subtypes of VGSCs through *in-situ* hybridization. We found that the expression of $Na_V1.2$, 1.3, 1.6 sodium channels were significantly up-regulated in P12 rat hippocampus. Interestingly, the expression of $Na_V1.5$, a well-known cardiac sodium channel, was also enhanced. Together, our data demonstrate that elevation of VGSCs during neonatal development in hippocampal neurons coincides with the maturation of neuronal functions.

Keywords: hippocampal neuron, voltage-gated na^+ channel, action potential

P7-9

Rapid astrocyte-dependent facilitation amplifies multi-vesicular release in hippocampal synapses

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Facilitation is a well-known form of short-term synaptic plasticity that enhances neurotransmitter release on timescales from tens to hundreds of milliseconds. It is widely believed to arise from an increase in residual presynaptic calcium that accumulates during elevated neural activity. However, recent studies have highlighted the potential role of astrocytes in modulating neurotransmitter release. Astrocytes, which are the most abundant glial cells in the central nervous system, have been shown to modulate synaptic transmission in a variety of ways, including regulating basal synaptic transmission, potentiating or depressing neurotransmitter release, and controlling the timing and direction of modulation.

To investigate the role of astrocytes in synaptic facilitation, we used combination of near-total internal reflection fluorescence (near-TIRF) imaging of single-vesicle release and vesicle-bound pH-sensitive indicator which indicates fusion of vesicles with plasma membrane expressed in hippocampal synapses in neuronal-astrocyte co-cultures.

The study confirmed the presence of release-dependent facilitation and showed that it is astrocyte-dependent, requiring dynamic neuron-astrocyte interaction. The study also found that this form of facilitation lasts several seconds and is detectable as early as 500 ms following a preceding release event. The study employed different algorithms including feed forward deep-learning to detect release events, confirming the presence of release-dependent facilitation. The release-dependent facilitation requires neuronal contact with astrocytes and astrocytic glutamate uptake by EAAT1. It is not observed in neurons grown alone or in the presence of astrocyte-conditioned media. This form of facilitation dynamically amplifies multi-vesicular release. Facilitation-evoked release events exhibit spatial clustering and have a preferential localization towards the active zone center. These results uncover a rapid astrocyte-dependent form of facilitation acting via modulation of multi-vesicular release and displaying distinctive spatiotemporal properties.

Keywords: synapse, astrocyte, EAAT1, deep learning

P7-10

Modulation of immune function by glutamatergic neurons in the cerebellar interposed nucleus via hypothalamic and sympathetic pathways

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Background: Our recent work has shown that the cerebellar interposed nucleus (IN) contains glutamatergic neurons that send axons directly to the hypothalamus. Here, we aimed to demonstrate modulation of cellular and humoral immunity by glutamatergic neurons in the IN by means of gene interventions of glutaminase (GLS), an enzyme for glutamate synthesis, and to reveal pathways transmitting the immunomodulation.
Methods: Rats were intraperitoneally immunized with bovine serum albumin (BSA) on day 1 following GLS-shRNA lentiviral vector or GLS lentiviral vector injections in bilateral IN and 6 days later, T and B lymphocyte percentages in peripheral blood mononuclear cells, cytokine

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levels in the supernatants of concanavalin A-stimulated lymphocytes and IgM level in the serum were detected; Meanwhile, glutamate content in the hypothalamus, norepinephrine content in the spleen and mesenteric lymph nodes, adrenocortical and thyroid hormone levels in the serum were determined.
Results: GLS silencing in the cerebellar IN decreased interleukin-2 and interferon-γ production, B-cell number, and IgM level in response to BSA. On the contrary, GLS overexpression caused opposite immune effects to GLS silencing. Simultaneously, GLS silencing reduced and GLS overexpression elevated glutamate content in the hypothalamus. In addition, immune changes caused by GLS gene interventions were accompanied by alteration in norepinephrine content in the spleen and lymph nodes.
Conclusion: These findings indicate that glutamatergic neurons in the cerebellar IN regulate cellular and humoral immune responses and suggest that such immunoregulation may be conveyed by cerebellar IN-hypothalamic glutamatergic projections and sympathetic nerves that innervate lymphoid tissues.

Keywords: interposed nucleus, glutamatergic neurons, cerebellar, humoral immunity, cellular immunity

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P7-11

The intracellular C-terminal domain of mGluR6 works as a signal for ER retention

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The metabotropic glutamate receptor 6 (mGluR6) is exclusively expressed at the dendritic tips of On-type bipolar cells, and exerts a pivotal role for signal processing in the On-pathway in the retina. However, the trafficking mechanisms of mGluR6 to dendrites of On-type bipolar cells have not been well elucidated. Since the intracellular C-terminal domain (CTD) of mGluR6 interacts with scaffold proteins and Gβγ subunits, it is likely that CTD of mGluR6 contributes to cell surface localization and intracellular signaling of mGluR6. We recently proposed that a cluster of basic residues within the mGluR6 CTD might serve as signals for endoplasmic reticulum (ER) retention (Rai et al. J. Neurochem. 2021). We herein examined whether the basic residues participated in mGluR6 intracellular trafficking, using 293T cell expression system with immunocytochemistry, immunoprecipitation and flow cytometry. In 293T cells, mGluR6 was distributed in the ER and localized on cell surface, while in the mGluR6 mutants with 15-, 16-, 19- and 20-amino acid deletions at the C-terminus of mGluR6, mGluR6 level was maintained in the ER but showed significant reductions in cell surface. The observed deficiencies in cell-surface localization were rescued by introducing alanine substitution at the basic residues. Surface-deficiency on the surface-deficient mutant was not rescued with co-expression of the surface-expressible constructs, including full-length mGluR6, although the full-length mGluR6 construct and the surface-deficient construct formed heteromeric complexes in co-expressing 293T cells. These results indicate that the basic residues in the mGluR6 CTD formed ER retention signals, which may prevent aberrant mGluR6 assemblies from being transported to the cell-surface.

Keywords: metabotropic glutamate receptor 6, ER retention motif, retina, surface expression, bipolar cell

P7-12

Starburst amacrine cells form gap junctions with other cell types in early postnatal stage of the mouse retina

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In the central nervous system, neuronal networks via gap junctions in late embryonic and early postnatal stages disappear until adulthood. On the other hand, in the retina, gap junctional couplings are used for signal processing in adulthood. Currently, starburst amacrine cells (SACs) are known to play an important role in the generation of retinal waves in the developmental stage and the formation of direction selectivity in the retina in adulthood, and recognized as a neuron which does not form gap junctional coupling in adulthood. However, it remains unclear whether SACs form gap junctional coupling during the developmental stage.
In the present study, therefore, we examined whether SACs form gap junctional coupling with neighboring cells at the developmental stage in the mouse retina using immunohistochemical, electrophysiological, and molecular biological techniques. When we injected Neurobiotin into SACs, many tracer-coupled cells were detected before eye-opening. The tracer coupled cells were RNA-binding protein with multiple splicing (RBPMS)-positive cells, retinal ganglion cells, but not SACs. Number of tracer-coupled cells was significantly reduced after eye-opening. In SACs, membrane capacitance at P9 was larger than the membrane capacitance after eye-opening. The membrane capacitance after eye opening was close to the membrane capacitance of single cell level of SACs, suggesting that gap junctional couplings observed at P9 disappear after eye opening. The reduction of membrane capacitance after eye opening was not affected by visual experience. At the mRNA level, connexin 43 expression before eye opening was significantly higher than that after eye opening in SAC. Block of gap junctional couplings by an application of meclofenamic acid (MFA) to SACs resulted in a significant decrease in membrane capacitance of SACs. In addition, the application of MFA reduced the frequency of retinal waves before eye opening. These results support the notion that SACs form gap junctions in early postnatal period, and inherently reduce the connection to neighboring cells during the development.

Keywords: starburst amacrine cell, cholinergic amacrine cell, gap junction, connexin, retina

P7-13

Activity-dependent regulation of tonic firing rate by TRPC3 channels in SNc dopamine neurons

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Pacemaker dopamine (DA) neurons in the substantia nigra pars compacta (SNc) display low-frequency tonic firing, but they are capable of generating high-frequency burst firing when stimulated by strong synaptic inputs. The firing rate of DA neurons not only determines the levels of ambient DA but also contributes to the intensity of bursts. Therefore, it is very important to investigate the mechanisms that regulate the tonic firing in SNc DA neurons. In this study, we demonstrate that robust or repetitive subthreshold stimulation of glutamatergic fibers innervating SNc DA neurons leads to an increase in tonic firing

rate by activating TRPC3 channels through a metabotropic glutamate receptors (mGluR1). This enhanced firing rate assists in generating more powerful bursts in SNc DA neurons. Application of DHPG, an agonist of mGluR1, induced a gradual and sustained elevation in tonic firing rates accompanied by an increase in calcium levels, following a transient inhibition caused by the release of stored calcium. Blocking TRPC3 channels with the pyr10 compound significantly diminishes the DHPG-induced calcium rises and eliminates the enhancement of tonic firing. In SNc DA neurons lacking TRPC3, the firing rate of DA neurons does not increase in response to DHPG or repetitive synaptic stimulation. Based on these experimental findings, we can conclude that afferent synaptic activities regulate the tonic firing and intensity of bursts through TRPC3 channels in a mGluR1-dependent manner in SNc DA neurons.

Keywords: dopamine neuron, TRPC3, mGluR1, substantia nigra compacta, spontaneous firing

P7-14

The exploration of the detecting methods for new psychoactive substances (NPS) using synaptic receptors

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The rapid emergence of a large number of NPS on the global drug market invades both public health and drug policy. Often, little is known about the adverse health effects and social harms of NPS, which face a considerable challenge for prevention and for health. There is a lack of scientific evidence to regulate drug policy for controlling NPS production. And anyone who easily buys NPS from social networks and hidden markets could be a big problem in South Korea and international society. In addition, behavioral complexity causes to take a long time to judge coming NPS. Therefore, the fast and accuracy that are able to quantify the neurotoxicity and addiction of NPS is required to provide scientific evidence. Here we introduced major synaptic channels, GluRL239Y, GluR1 desensitization mutant for detecting glutamate, GABAc for detecting gamma aminobutyric acid (GABA) and GPCR-activation–based DA ($GRAB_{DA2m}$) for detecting dopamine. To analyze whether NPS affects the excitability of neural membrane and synaptic glutamate release, we measured action potential and excitatory postsynaptic current (EPSC) by using a whole-cell patch clamp. Methamphetamine, cocaine, delta-8-tetrahydocdannabinol (THC) were used as the reference drugs and 1-(4-ethylphenyl)-N-[(2-methoxyphenyl)methyl]propan-2-amine (4-EA-NBOMe), Methyl (2R)-2-(3,4-dichlorophenyl)-2-[(2R)-piperidin-2-yl]acetate (3,4-DCMP), 5-(4-Biphenylylmehtyl)-N,N-dimethyl-1H-tetrazole-1-carboxamide (LY-2183240) and N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(cyclohexylmethyl)-3-(4-flurophenyl)-1H-pyrazole-5-carboxmide (AB-CHFUPYCA) were tested as NPS on *in vitro* synaptic channels. For the expression of GluR1LY and GABAc, the plasmids of GluR1LY and GABAc were transfected into the human embryonic kidney 293 (HEK 293) cells and incubated for 12-19h. THC and AB-CHFUPYCA induced 6% and 3% glutamate-mediated currents, suggesting THC and AB-CHFUPYCA modulate synaptic glutamate channels. But all drugs did not induce GABAc-mediated current. For the detection of dopamine, $GRAB_{DA2m}$ plasmids were transfected in primary cultured rat cortical astrocytes. Once 1 μM dopamine was treated to astrocytes expressing $GRAB_{DA2m}$, GFP transients were induced. 0.1 mM 4-EA-NBOMe and 1 mM Methamphetamine induced GFP transients are the same as 4 μM and 1~10 μM dopamine-induced GFP transients in average value, suggesting that 4-EA-NBOMe and methamphetamine can modulate dopamine channels. 4-EA-NBOMe and LY-2183240 increased the number of action potentials, suggesting changing intrinsic firing properties. 4-EA-NBOMe, LY-2183240, and AB-CHFUPYCA influence synaptic channels and increase neuronal excitability. These results provide scientific evidence for quantitative and fast neurotoxicity evaluation.

Keywords: new psychoactive substances (NPS), drugs, synaptic channels, neurotoxicity

P7-15

Bicarbonate permeability of synaptic GABAAR mediates neuronal excitation

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gamma-Aminobutyric acid type A receptors ($GABA_AR$s) are ligand-gated anion channels that principally mediate inhibitory neurotransmission. However, intensely activated synaptic $GABA_AR$s in adult neurons paradoxically produce neuronal depolarization and excitation. Although $[Cl^-]_i$ increases and resultant Cl^- efflux through $GABA_AR$s may play a role, precise mechanisms that underlie the GABAergic excitation remain largely elusive. Here we show that a prolonged and intense agonist stimulation dynamically increases the bicarbonate permeability (P_{HCO3}/P_{Cl}) of synaptic $GABA_AR$s, which contributes to neuronal excitation. Whole-cell current measurements in HEK 293T cells expressing synaptic (phasic) $a_1b_3g_{2L}$ GABAAR showed that an increment of stimulating GABA concentrations from 1 μM to 1 mM evoked an increase in P_{HCO3}/P_{Cl} from 0.14 to 0.53. However, the same stimulation did not alter the P_{HCO3}/P_{Cl} of extra-synaptic (tonic) $a_4b_3\delta$ and $a_5b_3g_{2L}$ $GABA_AR$s. In the granule cells of dentate gyrus expressing both synaptic and extra-synaptic $GABA_AR$s ($a_4b_3\delta$), synaptic $GABA_AR$s activation with a q-shape glass stimulator generated action potential in granule cells filled with HCO_3^- (20 mM). In contrast, extra-synaptic $GABA_AR$s activation with 4,5,6,7-tetrahydroisoxazolo (5,4-c) pyridin-3-ol (THIP, 500 μM) puffs did not induce action potential. These results indicate that dynamic increases in HCO_3^- efflux-mediated depolarization underlie GABAergic excitations, which may play key roles in both physiological and pathological conditions.

Keywords: GABA, bicarbonate, neuronal excitation

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P7-16

NR2D-subunit containing NMDARs recall in hippocampal GABAergic interneurons regulates E/I balance in epileptic hippocampus

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N-Methyl-D-Aspartate receptors (NMDARs) are ion channels involved in most of the excitatory transmissions in the central nervous system. They are typically composed of NR1 and NR2 (NR2A-D) receptor subunits, which are differentially expressed in brain regions throughout development. NR2D subunit-containing NMDARs gradually disappear during brain maturation, but they can be reactivated by pathophysiological stimuli in the adult brain. NR2D mRNA is selectively expressed in GABAergic interneurons but not in glutamatergic neurons in both developing and mature hippocampus. However, the role of NR2D-containing NMDARs in the hippocampal GABAergic interneurons in a mature brain remains unknown. We hypothesized that NR2D in GABAergic interneurons contributes to maintaining of the E/I (excitation/inhibition) balance during neuronal hyperexcitability conditions such as epilepsy. Our experiment explores the Mg^{2+}-resistant tonic NMDA current mediated by NR2D-containing NMDARs, which is generated in the hippocampal GABAergic interneurons of pilocarpine-induced epileptic mice but not in the control mice. Additionally, the tonic activation of NR2D-containing NMDA receptors in GABAergic interneurons regulates synaptic inhibition in hippocampal CA1 pyramidal neurons. Furthermore, we examined the role of NR2D in epilepsy progression after pilocarpine injection. Interestingly, transauricular electroshock-induced seizure activity was increased in pilocarpine-injected NR2D knockout (KO) mice compared to wild-type (WT) mice. Moreover, KO mice exhibited progressive death of hippocampal pyramidal neurons. At the same time, pilocarpine effects were minimal in WT mice. We demonstrate for the first time that NR2D-mediated Mg^{2+}-resistant tonic NMDA current in GABAergic interneurons regulates GABA inhibition in pyramidal neurons and strongly contributes to the compensatory shift of E/I balance in epileptic hippocampi.

Keywords: status epilepticus, NMDARs, NR2D, GABAergic Interneurons

P7-17

Role of IFN-γ expression in trigeminal ganglion neurons in orofacial neuropathic pain

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Background: Trigeminal nerve injury is known to cause severe persistent pain in the orofacial regions. Various molecules are released in the trigeminal ganglion (TG) neurons, and these contribute to enhancing TG neuronal activity, resulting in persistent orofacial pain. However, the detailed mechanism underlying persistent orofacial pain remains unclear. Recently, it has been reported that interferon-γ (IFN-γ) signaling in the trigeminal spinal subnucleus caudalis neurons is involved in facial mechanical allodynia caused by infraorbital nerve injury (IONI), whereas that in TG is unknown. In the present study, we investigated the role of IFN-γ signaling in the TG in facial mechanical allodynia after IONI.
Methods: The ION was partially ligated in male SD rats under deep anesthesia to develop a rat model of IONI. Rats receiving only ION exposure were defined as sham. TGs were removed 14 days after IONI, and IFN-γ and IFN-γ receptor (IFNGRα) expression were precisely analyzed in TG. IFNGRα antagonist or vehicle was continuously administered in the TG, and mechanical head-withdrawal threshold (MHWT) to the whisker pad skin was measured until day 14 after IONI. In addition, IFN-γ was continuously administered into the TG in naive rats, and MHWT was measured until day 14.
Results: MHWT was significantly reduced in the IONI rats compared with the sham rats. On day 7, IFN-γ expressed in TG neurons, and IFNGRα in satellite cells, and the amount of IFN-γ protein in TG was significantly increased in the IONI rats compared with sham rats. Following continuous administration of IFN-γ into the TG in naive rats, MHWT was reduced considerably.
Conclusion: These observations suggest that IFN-γ signaling via IFNGRα expressed on satellite cells in TG may be involved in facial mechanical allodynia caused by IONI.

Keywords: trigeminal nerve, trigeminal ganglion, infraorbital nerve injury, allodynia, IFN-γ

P7-18

Licochalcone a attenuates NMDA-induced synaptic degeneration

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Licochalcone A (Lico A), a flavonoid extracted from licorice root (Glycyrrhiza glabra), is known for its anti-inflammatory, anti-cancer, anti-oxidadant properties. In this study, we aimed to investigate the effects of licochalcone A on NMDA (N-methyl-D-aspartate)-induced neurotoxicity in cultured rat hippocampal neurons. Using a 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay, we assessed the viability of hippocampal neurons treated with NMDA and licochalcone A. Our results demonstrated a significant decrease in cell viability following NMDA treatment. However, when licochalcone A was treated at a concentration of 2.5ug/ml after NMDA exposure, we observed a significant increase in cell viability comparable to the control group. To investigate the effects of licochalcone A on NMDA-induced synapse loss, we quantified the number of synapses expressing postsynaptic density 95 (PSD95) using an imaging-based assay. The NMDA-treated group exhibited a significant reduction in PSD95 puncta compared to the control group. While the group treated with licochalcone A at 2.5ug/ml demonstrated a significant increase in PSD95 puncta compared to the NMDA group. Furthermore, we examined western blot analysis to analyze the level of phosphorylated mixed lineage kinase domain-like pseudokinase (P-MLKL), a key regulator of necrotopsis. The group treated with NMDA showed a significant increase in P-MLKL levels compared to the control group. In contrast, the group treated with NMDA followed by licochalcone A at 2.5ug/ml exhibited a significant decrease in P-MLKL levels, indicating a potential protective effect against NMDA induced neurotoxicity. Given that excessive glial cell activation can contribute to necroptosis, we performed immunostaining for glial fribrillary acidic protein (GFAP) as an astrocyte marker and ionized calcium-binding adaptor molecule 1 (IBA-1) as a microglial marker. The NMDA treated group showed a significant increase in GFAP intensity, while the licochalcone A-treated group at 2.5 ug/ml exhibited a significant decrease in GFAP intensity compared to the NMDA group. Similarly, IBA-1 intensity showed an increasing trend in NMDA group, whereas the licochalcone A- treated group demonstrated a decreasing trend.

Collectively, our findings suggest that licochalcone A exerts neuroprotective effects against NMDA-induced neurotoxicity in cultured rat hippocampal neurons. It shows potential in preserving synaptic integrity, preventing neuronal cell death, and modulating glial cell activaition. Licochalcone A may hold promise as a therapeutic agent for neuroprotection in conditions associated with excessive NMDA-mediated neurotoxicity, such as Status epilepticus.

Keywords: licochalcone A, NMDA, neuroprotection, necroptosis

P7-19

Activation of folate receptor 1, which was upregulated by high glucose, inhibited mitochondrial oxidative stress through Nrf2, thereby suppressing amyloidogenesis

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Diabetes is a critical risk factor for Alzheimer's disease and dementia occurrences, called type 3 diabetes. Previous meta-analysis studies have shown that folic acid (FA) levels and its receptor closely is associated with Alzheimer's disease and diabetes pathogenesis. However, the mechanism by which folic acid controls high glucose-induced amyloid-beta generation in neuronal cells remains poorly understood. Therefore, we investigated the regulatory effect of folic acid on high glucose-induced amyloidogenesis and its related mechanism. In our study, high glucose increased APP and BACE1 expressions and amyloid beta production. Folic acid treatment reversed high glucose-stimulated APP and BACE1 expressions and mitochondrial reactive oxygen stress accumulation. In addition, high glucose decreased Nrf2 expression, recovered by folic acid. We further found that high glucose increased folate receptor 1 (FOLR1) mRNA expression, reduced by OGT inhibitor pretreatment. 5-methyltetrahydrofolate (5-MTHF) treatment did not significantly affect the high glucose-induced APP and BACE1 expressions. Thus, we suggest that folic acid affects amyloidogenesis via the FOLR1 pathway. Animal experiments were performed to evaluate the effect of folic acid in streptozotocin-induced diabetic mice. In brain tissues, it was confirmed that high glucose-increased APP, BACE1, and FOLR1 expressions. In conclusion, activation of FOLR1 by folic acid suppresses mitochondrial oxidative stress and amyloidogenesis in neuronal cells under high glucose.

Keywords: SH-SY5Y, Folic Acid, FOLR1, Diabetes Mellitus, STAT3

P7-20

IL-17A exacerbates neuroinflammation and neurodegeneration by activating microglia in rodent models of Parkinson's disease

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Neuroinflammation has been involved in pathogenesis of Parkinson's disease (PD), a chronic neurodegenerative disease characterized neuropathologically by progressive dopaminergic neuronal loss in the substantia nigra (SN). We recently have shown that helper T (Th)17 cells facilitate dopaminergic neuronal loss in vitro. Herein, we demonstrated that interleukin (IL)-17A, a proinflammatory cytokine produced mainly by Th17 cells, contributed to PD pathogenesis depending on microglia. Mouse and rat models for PD were prepared by intraperitoneal injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or striatal injection of 1-methyl-4-phenylpyridinium (MPP +), respectively. Both in MPTP-treated mice and MPP + -treated rats, blood–brain barrier (BBB) was disrupted and IL-17A level increased in the SN but not in cortex. Effector T (Teff) cells that were adoptively transferred via tail veins infiltrated into the brain of PD mice but not into that of normal mice. The Teff cell transfer aggravated nigrostriatal dopaminergic neurodegeneration, microglial activation and motor impairment. Contrarily, IL-17A deficiency alleviated BBB disruption, dopaminergic neurodegeneration, micro-glial activation and motor impairment. Anti-IL-17A-neutralizing antibody that was injected into lateral cerebral ventricle in PD rats ameliorated the manifestations mentioned above. IL-17A activated microglia but did not directly affect dopaminergic neuronal survival in vitro. IL-17A exacerbated dopaminergic neuronal loss only in the presence of microglia, and silencing IL-17A receptor gene in microglia abolished the IL-17A effect. IL-17A-treated microglial medium that contained higher concentration of tumor necrosis factor (TNF)-α facilitated dopaminergic neuronal death. Further, TNF-α-neutralizing antibody attenuated MPP + -induced neurotoxicity. The findings suggest that IL-17A accelerates neurodegeneration in PD depending on microglial activation and at least partly TNF-α release.

Keywords: Interleukin-17A, Parkinson's disease, microglia, dopaminergic neurons, neuroinflammation

P7-21

WITHDRAWN

P7-22

Role of astrocyte-secreted lipocalin-2 in a mouse model of hepatic encephalopathy

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Background: Acute liver failure is a devastating consequence of hepatotoxic liver injury that can lead to the development of neurological complications called hepatic encephalopathy (HE) that is accompanied by high mortality. As the most abundant cell type in the central nervous system (CNS), astrocytes are recognized as playing a central role in the pathophysiology of HE. Based on the transcripts analysis from astrocyte purified from the brainstem of a mouse model of HE showed that gene expression of lipocalin-2 (LCN2) was significant increase. Our laboratory demonstrated that neuronal death in the rostral ventrolateral medulla (RVLM), a key neural substrate that maintains blood pressure and sympathetic vasomotor tone, leading to baroreflex dysregulation, is causally related to death in animal models of HE. Therefore, the aim of this study was to delineate the role of LCN2 between astrocytes and neurons in the RVLM during HE.
Methods: In this study, an azoxymethane (AOM; 100 μg/g, ip)-induced acute liver failure model of HE in C57BL/6 mouse was used. Primary neuronal culture and primary astrocyte culture prepared from postnatal day 1 mouse pups were carried out to proof-of-concept experiments.
Results: We demonstrated that LCN2 receptor (LCN2R) is present in RVLM neurons and observed that LCN2 protein levels were significantly

increased after administration of AOM, and we also observed that mitochondrial dysfunction leads to apoptosis in RVLM during HE. Our *in vitro* results revealed that treatment with recombinant LCN2 protein reduces cell viability and ATP production in cultured neurons. However, knockdown of LCN2R reversed the reduction of cell viability caused by LCN2. To mimic the secretion of LCN2 by reactive astrocytes in animals, primary astrocytes were pretreated with LPS (100 ng/ml) plus IFN-γ (50 U/ml) for 24h to stimulate LCN2 secretion, and then astrocyte-conditioned media were collected and added to cultured neurons. Our results further revealed that astrocyte-derived LCN2 promotes neuronal death.

Conclusion: Our results suggested that astrocyte-secreted LCN2 induces neuronal death in the RVLM through disruption of ATP production. The detailed cellular mechanism by which LCN2/LCN2R signaling disrupts mitochondrial bioenergetic function and triggers neuronal apoptosis will be investigated in the future.

Keywords: acute liver failure, LCN2, cell viability, mitochondrial dysfunction

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WITHDRAWN

P7-24

Alleviative effect of pinostrobin from *Boesenbergia rotunda* (L.) against scopolamine-induced the alteration of glial cells and glutamate receptor in the medial prefrontal cortex

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Scopolamine that is a muscarinic receptor antagonist can disrupt cholinergic neurotransmission resulting in memory and mood disturbances. Natural ingredients are capable of growing evidence on the regulation of neurotransmission. Accordingly, the aim of the present study was to examine the effect of pinostrobin from *Boesenbergia rotunda* (L.) in reversing scopolamine induced structural remodeling of glial cells and glutamate receptor that involved in synaptic transmission in the medial prefrontal cortex of rats. Twenty-four male Wistar rats were randomly divided into four groups (n = 6): group 1 received vehicle as control, group 2 received vehicle + scopolamine (3 mg/kg, i.p.), group 3 received pinostrobin (40 mg/kg, p.o) + scopolamine, and group 4 received donepezil (5 mg/kg, p.o) + scopolamine. Rats were treated once daily for 14 days. During the final 7 days of treatment, a daily injection of scopolamine was administered. The changes in expression of microglia and astrocytes in the prefrontal cortex were measured by immunohistochemical analysis. These findings demonstrated that scopolamine-induced rats showed a significant increase in ionized calcium binding adapter molecule 1 (Iba-1) immunoreactivity as a marker of microglia, and glial fibrillary acidic protein (GFAP) as a marker of astrocytes in the prefrontal cortex. Treatment with pinostrobin at a dose of 40 mg/kg significantly decreased the activation of microglia and astrocytes by reducing the immunoreactivity of Iba-1 and GFAP. In addition, we also determined the alteration of glutamate receptor (GluR1) within the prefrontal cortex. The result showed that pinostrobin could reverse scopolamine-induced the elevation of GluR1 expression involved in regulating synaptic transmission. To our knowledge this current study is the first to evaluate the alleviative effect of pinostrobin on the glial cells disturbances in the prefrontal cortex following scopolamine-induced the disruption of synaptic transmission leading to memory deficit.

Keywords: pinostrobin, scopolamine, glial cell, glutamate receptor, prefrontal cortex

P7-25

Structural alterations of glial cells associated with the disturbances of glutamatergic signaling within the hippocampus following acute stress and chronic restraint stress

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Chronic stress that is a crucial factor profoundly affects the structure and function of neuronal and glial cells related to mood and cognitive disorders. Glial cells including microglia and astrocytes have now been demonstrated to play an important role in contributing to neurotransmission and other essentially neuronal activities such as learning and memory. Previous studies demonstrated that chronic stress could alter the glial morphology, affecting synaptic transmission. Therefore, the present study aimed to examine how acute and chronic stress disrupt the structural remodeling of microglia and astrocytes as it involved in alteration of glutamatergic signaling. Twenty-four male Sprague–Dawley rats were randomly divided into two main groups: acute stress, chronic stress, and appropriate control groups. Acute stress-treated rats were exposed to a single stress (6 h/day), whereas chronically stressed rats were exposed to stress for 21 days (6 h/day). After the last exposure to stress, rats were perfused and immunohistochemistry analysis of microglia (Iba-1), astrocytes (GFAP), astrocytic glutamate transporter (EAAT1), astrocytic glutamate metabolism (GS), and proteins involved in glutamatergic regulation (GluA1, GluA2, GluN1, and vGluT1) was determined. The results showed that acute stress significantly decreased the expression of Iba-1, GFAP, EAAT1, GluA1, GluA2, GluN1, GS, and vGluT1 within the hippocampus. On the other hand, chronic stress-exposed rats showed a significant increase in the immunoreactivity of Iba-1, EAAT1, GluA2, and vGluT1 as well as a reduction in GFAP, GluA1, and GluN1 expression. However, chronic stress had no effect on GS level following chronic stress exposure. Taken together, our findings suggested that the exposure to both acute stress and chronic stress could induce the morphological alterations of microglia and astrocytes associated with the disturbances of glutamatergic signaling.

Keywords: microglia, astrocytes, acute stress, chronic stress, hippocampus

P7-26

Astrocytic PAR1 and mGluR2/3 control synaptic glutamate time course at hippocampal CA1 synapses

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Astrocytes play an essential role in regulating synaptic transmission. This study aimed to describe a novel form of modulation of excitatory synaptic transmission in the mouse hippocampus by G protein-coupled receptors (GPCRs). Despite our previous data on astrocytic glutamate release via protease-activated receptor-1 (PAR1) activation, the regulatory mechanism of glutamate remains controversial. Through electrophysiological analysis and modeling, we discovered that PAR1 activation consistently increases the concentration and duration of glutamate in the synaptic cleft. This effect was not due to changes in the presynaptic glutamate release, postsynaptic AMPA function, or glutamate transporters. Furthermore, we demonstrated that blocking group II metabotropic glutamate receptors (mGluR2/3) abolished PAR1-mediated regulation of synaptic glutamate concentration. The activation of mGluR2/3 causes glutamate release through the TREK-1 channel without affecting Ca^{2+} responses in hippocampal astrocytes. In conclusion, astrocytic GPCRs engage in a novel regulatory mechanism to shape the time course of synaptically released glutamate in the brain.

Keywords: astrocyte, PAR1, mGluR2/3, glutamate, synapses

P7-27

Visualizing astrocyte-neuron contacts with eGRASP

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Astrocytes communicate with neurons through their close contact and actively participate in neuronal processes and synaptic plasticity. However, the identification and examination of astrocyte-neuron contacts have been technically challenging. In this study, we have developed a novel tool, astrocyte-eGRASP (enhanced green fluorescent protein reconstitution across synaptic partners), to visualize astrocyte-neuron contacts and investigate the structural basis of their interaction. Our newly developed tool successfully labeled astrocyte-neuron contacts both in vitro and in vivo. By utilizing astrocyte-eGRASP, we discovered an increase in astrocyte-neuron contact in engram cells following learning. These findings provide further evidence supporting the significance of astrocyte-neuron communication in the processes of learning and memory.

Keywords: astrocyte, hippocampus, dual-eGRASP, learning and memory, engram

P7-28

Role of astrocyte-specific TTF-1 in high-fat-diet-induced hypothalamic inflammation

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Hypothalamus serves as a central hub for the regulation of energy homeostasis, overseeing feeding behavior and energy metabolism. Recent studies have highlighted the significant role of hypothalamic inflammation as a primary contributor to obesity, leading to reactive gliosis and an associated upsurge in proinflammatory cytokines. TTF-1 plays a crucial role in maintaining overall energy homeostasis by regulating the expression of hypothalamic neuropeptides that plays a vital role in controlling energy balance. The precise function of TTF-1 in hypothalamic astrocytes remains uncertain. Therefore, this study aimed to explore the role of TTF-1 in hypothalamic astrocytes and its involvement in high-fat-diet (HFD)-induced inflammatory responses. Our findings revealed a significant reduction in *Ttf-1* mRNA levels within hypothalamic astrocytes of animals subjected to HFD feeding. To investigate deeper into the function of TTF-1 in astrocytes, we developed knockout (KO) mice with astrocyte-specific TTF-1 deficiency. Remarkably, the KO mice exhibited increased reactive gliosis and upregulated expression of inflammatory cytokines compared to their wild-type counterparts. Moreover, during HFD feeding, the KO mice displayed significantly elevated food intake and greater weight gain in comparison to wild-type mice. These findings strongly suggest that TTF-1 in hypothalamic astrocytes plays a pivotal role in modulating inflammatory responses by regulating reactive gliosis and the expression of proinflammatory cytokines induced by HFD.

Keywords: hypothalamus, astrocyte, reactive gliosis, TTF-1, high fat diet

P7-29

Mancozeb induces atrophy through inhibition of store-operate Ca^{2+} Entry (SOCE) in astrocyte

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Store-operated Ca^{2+} entry (SOCE) is a major Ca^{2+} signaling by the interaction of Orai1 of the plasma membrane and STIM1 of endoplasmic reticulum (ER) and it is maintaining the intracellular Ca^{2+} homeostasis in especially astrocytes. However, the inhibition of Orai1-mediated SOCE in astrocytes is unknown in astrocyte-related functions and behavioral changes. Here, oral administrations 0.5 μg/kg/day Mancozeb, a fungicide, for 4 weeks induce atrophy of glial fibrillary acidic protein (GFAP), a marker of astrocytes, in the various brain regions. Also, oral administrations 30 μg/kg/day (acceptable daily intake, ADI) Mancozeb for 1 week reduce GFAP expression and shows hyper-locomotor activity of open fields test. Interestingly, SKF-96365, a non-specific SOCE antagonist, induces atrophy of GFAP like Mancozeb *in vitro* primary astrocyte culture. Mancozeb, reduces SOCE by comparing SKF-96365 and HC-03003, a TRPA1 antagonist, as negative control. In addition, Mancozeb-inhibited SOCE depends on short hairpin RNA (shRNA) of Orai1 and STIM1, in contrast to SOCE in the presence of scramble shRNA and TRPA1, suggesting that Mancozeb-inhibited SOCE requires Orai1 and STIM1. In the pretreatment of Mancozeb, MRS2365, a specific P2Y1 receptor agonist,

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reduces the peak of Ca^{2+} transients because of the lack of Ca^{2+} store of ER. Mancozeb induces atrophy by the reduced Ca^{2+} store of ER and hyper-locomotor activity. This is the first report that the inhibition of SOCE causes to atrophy of astrocyte, which is able to change general behaviors.

Keywords: Mancozeb, SOCE, astrocyte, atrophy

P7-30

Traumatic brain injury causes cognitive deficits via modulating gut microbiota and neuroinflammation and microglia responses

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Background: Epidemiologic studies have shown that experiencing a traumatic brain injury (TBI) in early or midlife is associated with an increased risk of dementia in later life. The gut-brain axis and its potential role in neurodegenerative diseases is a complex and emerging field of research. Further studies are needed to elucidate these relationships and to explore possible therapeutic interventions that target the gut microbiota to help prevent or mitigate cognitive deficits and dementia after TBI. The major aim of the present study is to elucidate the association of gut microbiota, serum cytokines/chemokines, and microglia responses in TBI-induced cognitive deficits.
Methods: We compared the microbiomes of repetitive TBI (rTBI) rats and sham control rats at different stages of rTBI progression (from day 3 to month 1), using 16S ribosomal RNA (rRNA) gene amplicon sequencing. The serum cytokines and chemokines arrays were conducted to identify systemic inflammation. We also analyzed the correlation between gut microbiota abundance and cytokines/chemokines expression at acute and chronic rTBI progression stages. We characterized the phenotypes of microglia involved in amyloid-beta (Aβ) genesis after neuronal damage using a microglia-neuron co-cultured system.
Results: Data have shown that repetitive Traumatic Brain Injury (rTBI) induces progressive amyloid-beta (Aβ) accumulation as well as increased beta-site APP cleaving enzyme 1 (BACE1) activation in the rat hippocampus and cortex. These changes were also correlated with motor and cognitive impairment in a time-dependent manner. Using a microglia-neuron co-culture system, we found that stretch injury-induced Aβ accumulation, axonal injury, and reduced viability in cultured primary rat cortical neurons in vitro. Stretch injury or injured BV2 cells caused Aβ accumulation, synaptic abnormalities, and reduced viability in cortical neurons; however, intact BV2 cells protected against stretch-induced damage. Histopathology data demonstrated rTBI-induced subepithelial lifting, sloughing, blunted and clubbed villi, mucosal edema in the villi, tight junction protein (claudin-1 and ZO-1) disintegration, and epithelium hyperpermeability. We also profiled the gut microbiota of rats before rTBI, on day 3, and on day 28 post-rTBI via 16S rRNA sequencing. Numerous correlations existed between gut bacteria, aberrant cortical contusion, and cytokine expression. Co-occurrence network analysis revealed two unique gut-brain axes in rats: (1) increased intestinal o_Lactobacillales and o_Clostridiales downregulated VEGF and worsened brain contusion; (2) decreased intestinal o_Bacteroidales upregulated CXCL7 and CX3CL1 and inflamed the microglia.
Conclusion: We suggested that the observed gut microbiota alteration during brain trauma progression might be associated with neuroinflammation.

Keywords: traumatic brain injury, cognitive impairment, microbiome, immune responses

P7-31

Assessment of dendritic growth in transwell co-culture of sympathetic neurons with homotypic and heterotypic glia

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As the rat grows postnatally, there is dramatic increase in both neurite length and complexity. In autonomic ganglia, the number of synaptic inputs made by the innervating axons is dependent on the size of post-synaptic dendritic arbor. A previous study has shown that the dendritic growth is enhanced when sympathetic neurons were cocultured with endogenous ganglionic non-neuronal cells. However, our preceding study has revealed that both homotypic and heterotypic glia feeder-layers did not support the dendritic growth of sympathetic neurons in autaptic culture. From these unexpected results, we speculated that the glial feeder-layer might act as a physical resistance to neurite growth. Accordingly, in the current study, we tested transwell co-culture system in order to avoid any physical contacts between neurons and glial cells. In this regard, either astrocytes or autonomic glial cells were plated in transwell inserts (pore size: 0.4 μm) of 6-well plates at the cell density of 3×10^4 /well. The principal neurons of the superior cervical ganglia (SCG) from neonatal rats (P0-P2) were plated onto the agarose-coated coverslips which were stamped with growth-permissive substrates. Then, the neuron containing coverslips were transferred to the low compartment of transwell 6-plate system. Both types of cells were maintained in MEM supplemented with nerve growth factor and ciliary neurotrophic factor. After two-weeks of co-culture, we performed immunocytochemistry using an antibody against microtubule-associated protein 2 (MAP2) and quantified the dendritic growth using the ImageJ software. Compared with monoculture and direct co-culture using the glial feeder-layer, the transwell co-culture system significantly promoted the dendritic growth in the SCG neurons. There was no significant difference in the dendritic growth promoting effects between the homotypic and heterotypic glial cells. Taken together, these findings suggest that some glia-derived factors enhance dendritic growth in the sympathetic neurons in autaptic culture. Now, we are under investigation to see if the glia exerts stimulatory effects on autaptic synapse formation in the transwell co-culture.

Keywords: autaptic culture, autapse, autonomic ganglia, dendrite, transwell co-culture

Poster

P7-32

The inwardly rectifying potassium channel, Kir4.1-mediated calcium signaling in the satellite glial cells of sympathetic ganglia under hypokalemic condition: its molecular mechanism and functional consequence

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The glia-specific inwardly rectifying potassium channel Kir4.1 is activated in the resting state when there is a K^+ gradient, and buffers extracellular K^+ around neurons during neuronal excitation to control cell excitability. In the satellite glial cells (SGCs) of rat sympathetic ganglia, the Kir4.1 was also expressed and age-dependently upregulated. A previous study has shown that the Kir4.1 mediates Ca^{2+} entry into the astrocytes in response to low extracellular K^+ concentration although its molecular mechanism and functional consequence remain unknown. Thus, in the present study, we firstly tested whether the Kir4.1 mediates the same Ca^{2+} signaling in the SGCs as the one observed in the astrocytes. In this regard, sympathetic superior cervical ganglia were partially digested to produce the principal neurons attached with the SGCs. Ca^{2+} imaging analysis showed that the SGCs, but not the neurons, exhibited a large cytosolic Ca^{2+} increase in response to bath application of low K^+ (<2 mM) solution. The low $[K^+]_o$-induced $[Ca^{2+}]_i$ increase was abolished by Ba^{2+} (0.1 mM) and removal of external Ca^{2+}. Among different Kir channels including Kir1.1, Kir4.1, Kir4.2, Kir5.1, Kir6.1, and Kir6.2 transiently overexpressed in HEK293 cells, only the Kir4.1 mediated the low $[K^+]_o$-induced $[Ca^{2+}]_i$ increase. Bath application of FCCP (1 μM) depolarized the SGCs, which negated the low $[K^+]_o$-induced $[Ca^{2+}]_i$ increase, suggesting that hyperpolarization of the plasma membrane potential may drive Ca^{2+} entry through some Ca^{2+}-permeable ion channels such as transient receptor potential (TRP) channels. Interestingly, non-specific TRP channel blockers (La^{3+} and ruthenium red) or TRPC3-specific blockers (Pyr3 and Sar7334) significantly reduced the low $[K^+]_o$-induced $[Ca^{2+}]_i$ increase in the SGCs. Consistent with these observations, overexpression of TRPC3 enhanced the Kir4.1-mediated calcium signaling in HEK293 cells. On the other hand, low $[K^+]_o$ evoked release of S100ß, an SGC-specific calcium binding protein which may buffer the Ca^{2+} outside the neurons to increase cell excitability. Overall, these data suggest that the Kir4.1 hyperpolarizes the SGCs in response to low $[K^+]_o$, which may allow Ca^{2+} entry via the TRPC3 channels. The calcium signaling cause release of S100ß from the SGCs which may increase cell excitability for readjusting the K^+ gradient to normal. At present, we are under investigation of the physiological links between Kir4.1 and TRPC activation in the SGCs of sympathetic ganglia.

Keywords: satellite glial cell, hypokalemic, excitability, transient receptor potential, TRPC3

P7-33

Acute stress decreases cerebellar tonic inhibition and enhances motor function

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Stress can have both positive and negative effects, facilitating concentration but impairing memory. During stressful situations, the adrenal cortex in the Hypothalamic-Pituitary-Adrenal Axis (HPA) releases increased amounts of glucocorticoids, including corticosterone (CORT). In a study conducted by Woo et al. (PNAS, 2018), it was found that astrocytic tonic GABA modulation regulates motor coordination. Building on this discovery, we exposed mice to various acute stressors, such as acute CORT intraperitoneal injection and subjected them to forced swimming. Following the forced swim stress, mice showed an enhanced latency to fall score in the rotarod test, indicating that acute stress enhances motor coordination. Furthermore, mice treated with selegiline, an MAOB inhibitor, or the MAOB knock-out mice, which shows reduced tonic GABA release, both exhibited enhanced motor coordination compared to the WT controls, while there was no further enhancement under stress condition. These results indicate that MAOB-dependent tonic GABA inhibition is critical for the stress-induced motor coordination enhancement**.** Through an electrophysiological study, we observed a significant reduction in tonic GABA current after CORT injection and forced swim stress. To simulate this data, we performed ex vivo acute CORT administration, resulting in a 43.88% decrease in cerebellar tonic GABA current. Furthermore, acute CORT treatment resulted in a 28.39% reduction in the full-$GABA_A$ receptor activation current by GABA, suggesting that the decline of tonic GABA current after acute CORT treatment can be a complex mixture of extrasynaptic $GABA_A$ receptor inhibition and astrocytic tonic GABA release decline. The MAOB knock-out mouse also showed a decline in tonic GABA, supporting that CORT treatment inhibits $GABA_A$ receptors. By normalizing $GABA_A$ receptor inhibition portion data, we estimated a contribution of astrocytic tonic GABA release decline at 15.49%, dissecting the CORT effect into both extrasynaptic $GABA_A$ receptor and astrocytic tonic GABA release. Our observations not only revealed enhanced motor function by the rotarod test but also demonstrated subtle changes in mouse behavior using state-of-the-art tools like the AVATAR behavior recorder and SUBTLE analysis. Based on these data, we propose that acute stress enhances motor function by decreasing cerebellar tonic inhibition, highlighting the stress response of astrocytes.

Keywords: stress, corticosterone, tonic GABA, motor coordination, astrocyte

P7-34

Correlations of molecularly defined cortical interneuron populations with morpho-electric properties

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Cortical interneurons can be categorized into distinct subtypes based on multiple modalities; however, it remains unclear to what extent these modalities are correlated. Here, we utilized patch-clamp single-cell RT-PCR (Patch-PCR) to investigate the correlations between mo-

lecular marker expressions and morpho-electric properties of over 600 layer 5 (L5) interneurons in the mouse somatosensory cortex (S1). Based on extracted morpho-electric features and differential expressions of neurochemical markers and transcriptomic signatures, we identified 11 morpho-electric subtypes (M/E types), 9 neurochemical cell groups (NC groups) and 20 transcriptomic cell groups (TC groups). We found that cells in NC groups and TC groups typically comprised several M/E types, yet combinatorial expression of certain neurochemical markers and expression levels of specific transcriptomic signatures were statistically correlated to given M/E types. Moreover, we found that, at the subclass level (Pvalb, Sst, Vip and Non-Vip), most interneurons exhibited distinct morpho-electric properties, and this distinction was relatively weak between individual TC groups. Similar results were also obtained in the primary visual cortex (V1) and motor cortex (M1) using recently published Patch-seq data. Interestingly, a significantly stronger correlation between morpho-electric properties and TC groups in V1 compared to S1 and M1 was observed. Systematic comparison of TC groups between these brain areas suggested that, compared to V1, S1 interneurons were morpho-electrically more similar to M1. Together, this study revealed a complex multimodal correlation landscape across different cortical areas.

Keywords: cortical interneuron, transcriptomic signatures, morpho-electric properties, cortical areas

Poster

P7-35

Beta frequency dynamics in the frontal cortex and neuropharmacological potential of indian cork flower tea during thiopental-induced sleep in mice

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There is a general relationship between lower high-frequency brain activity and decreased sense of arousal. However, benzodiazepines increase dominant beta activity (13-30 Hz) in the cortex associated with a compensatory mechanism that allows behavioral activation during sedation. Indian cork (*Millingtonia hortensis*) has a very pleasant and rich floral scent and is traditionally used in Southeast Asia to treat insomnia. In this study, India cork tree flower tea was tested for its anxiolytic and sedative effects on male ICR mice. A thiopental-induced sleep model was used to test for drug effects on GABA-A receptors without increasing beta modulation. Before thiopental (20 mg/kg, i.p.) injection, 10 ml/kg distilled water feeding, 1 mg/kg diazepam intraperitoneally or 2 mg/kg Indian cork flower tea feeding was administered to animals for 30 minutes. Electroencephalogram (EEG) was recorded from implanted dural surface electrodes in the frontal cortex. Thiopental sleep induction conducted using diazepam was shown to significantly enhance beta oscillations. A significant effect was also observed on beta activity over a period with Indian cork flower tea. To summarize, taking Indian cork flower tea before thiopental sleep induction could cause neural synchronization within the cortical structure, effectively inducing sleep as diazepam increases GABA-A receptor activity.

Keywords: sleep, EEG, benzodiazepines, thiopental, GABA-A receptor

P7-36

Lateral part of the interstitial nucleus of the anterior commissure (IPACL) CRF neurons promote wakefulness in mice

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Sleep and wakefulness are regulated by a complex interplay of various factors. Among these, stress has been identified as a leading cause of insomnia. Corticotropin-releasing factor (CRF) peptide plays a crucial role in the stress response. We previously reported that CRF-producing neurons in the paraventricular nucleus (PVN) contributed to the promoting wakefulness. Besides the PVN, CRF neurons are also highly distributed in other brain areas, such as the inferior olivary nucleus bed nucleus of the stria terminalis, lateral part of the interstitial nucleus of the anterior commissure (IPACL), and central amygdala. However, it is unclear whether CRF neurons in these areas are involved in sleep regulation.
Our c-Fos staining studies indicated that the novel environment activated the IPACL-CRF neurons. Therefore, we focused on IPACL-CRF neurons. To understand the role of IPACL-CRF neurons in sleep, designer receptors exclusively activated by designer drugs (DREADD) proteins were expressed by virus injection, and electroencephalography (EEG) and electromyography (EMG) were used to assess vigilant states. Activation of IPACL-CRF neurons increased wakefulness. Inhibition of these neurons did not affect sleep/wakefulness, but attenuated novel-environment-induced wakefulness. These results suggest that IPACL-CRF neurons promote wakefulness in novel environments. Further experiments are necessary to clarify the function of IPACL-CRF neurons.

Keywords: CRF, sleep, novel environment

P7-37

Significance of lateral habenula glutamate projection and miR-199a-3p/NEDD4 signaling on methamphetamine addiction

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Drug addiction encodes a chronic disorder with frequent relapse characteristics, could readily trigger physical and psychological dependence. Upon drug withdrawal, patients usually experience compulsive drug-seeking and severe withdrawal syndromes that engage further drug taking. Recently, several reports indicated the significance of miRNA and prefrontal glutamate on the development of neuroplasticity and behavioral manifestation, including the behavioral sensitization and conditioned place preference (CPP), two notable animal models for evaluating drug addiction. Therefore, the first aim of this study is to explore the altered miRNAs in methamphetamine (METH) sensitization. METH-sensitized mice received systemic daily METH (2 mg/kg, i.p.) treatment for 8 consecutive days. Brain tissues of mice nucleus accumbens (NAc), infralimbic cortex (IL), and prelimbic cortex (PL) were dissected and stored in freezer at -80°C. We found several miRNA species in these brain area were altered. Of those miRNAs, we consistently

found the miR-199a-3p expression increased significantly in the NAc of METH-sensitized mice. In addition, we selected 11 mRNAs that could be targeted by miR-199a-3p and measured their amount of expression in the NAc of behaviorally sensitized mice. Of which, NEDD4 mRNA and protein were significantly reduced in the NAc of METH-sensitized mice, matched with the change of miR-199a-3p. Next, we intent to explore if brain area of lateral habenula (LHb) would be involved in the extinction phase of METH-CPP since previously we found IL associated with an aversive stimuli and would send projection to LHb. Therefore, we speculate that neural projection from IL to LHb and LHb to ventral tegmental area (VTA) may initiate an extinguishing signal to suppress METH-CPP. Via optogenetic manipulation, we found inhibition of LHb-to-VTA or IL-to-LHb glutamate activity could significantly disrupt the extinction of METH-CPP. Further, via IHC, we confirmed that after the METH-CPP extinction test, numbers of c-Fos+ neurons in the LHb was significantly reduced when mice received photo-stimulation, indicating that NpHR indeed inhibited the neural activity. We also found tyrosine hydroxylase activated (pTH/S34) in the VTA after the glutamate neurons of IL or LHb was inhibited by photo-stimulation. On the other hand, GluR1 phosphorylation in parvalbumin+-GABA interneuron was significantly decreased in the LHb and VTA. Altogether, we demonstrate that miR-199a-3p/NEDD4 as well as IL-to-LHb and LHb-to-VTA neural circuits play a significant role in the progress of METH addiction and extinction.

Keywords: methamphetamine, lateral habenula, glutamate, miR-199a-3p, NEDD4

P7-38

Excitatory paths between inhibitory and pyramidal neurons enable network reverberation and epileptogenesis

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Epileptogenesis typically involves excessive reverberating activities or "afterdischarges" outlasting the initial trigger in the telencephalic networks. Theoretically, a "network-intrinsic" persistent excitatory drive after the cessation of the primary trigger should be existent for long-lasting changes in activities, but has not been defined. We found that this missing link is provided by dynamically deployed gap junctions between inhibitory and excitatory pyramidal neurons (IN and PN). This mechanism enables excitatory paths on top of inhibitory signals, a novel and crucial action turning the basic negative feedback design of a reciprocally innervating IN-PN circuitry into a positive feedback one to sustain long-lasting reverberations. These gap junctions are assembled and decomposed, or engaged in and disengaged from function, according to the spatiotemporal scope of network excitation. This crafts a novel form of context-dependent neural plasticity at the telencephalic level. The system therefore could respond differently to an external trigger based on the past experience and concurrent performance, constituting a macroscopically ordered but microscopically stochastic process underlying a wide spectrum of neurobiological and neuropathological manifestations including epileptogenesis.

Keywords: electrical synapse, epileptogenesis, rhythmogenesis

P7-39

A hypothalamus-habenula circuit regulates psychomotor responses induced by cocaine

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Administration of cocaine increases synaptic dopamine levels by blocking dopamine reuptake and leads to increased locomotor activity and compulsive drug-seeking behavior. It has been suggested that the lateral hypothalamus (LH) or lateral habenula (LHb) is involved in drug-seeking behaviors. To explore the role of the LH and the LHb in cocaine-induced psychomotor responses, we tested whether modulation of the LH or the LH-LHb circuit affects cocaine-induced locomotion and 50 kHz ultrasonic vocalizations (USVs). Cocaine-induced locomotor activity, 50 kHz USVs and dopamine release were suppressed by the activation of the LH with PEPA, an AMPA receptor agonist. When the LH was inhibited by microinjection of GABA receptor agonists mixture prior to cocaine injection, the cocaine's effects were enhanced. Furthermore, optogenetic activation of the LH-LHb circuit attenuated the cocaine-induced locomotion, while optogenetic inhibition of the LH-LHb circuit increased it. In vivo extracellular recording found that the LH sent a glutamatergic projection to the LHb. These findings suggest that the LH glutamatergic projection to the LHb plays an active role in the modulation of cocaine-induced psychomotor responses.

Keywords: lateral hypothalamu, lateral habenula, cocaine behavior, optogenetics, locomotion

P7-40

Transmittance measurements of the extremely low birth weight infant's head by near-infrared time-resolved spectroscopy

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Background: Monitoring extremely low birth weight infants (ELBWIs) using near-infrared spectroscopy (NIRS) is typically obtained from the forehead in the reflectance mode, which relies on a source and detection probe. However, as the surface of the cerebral tissue is covered in cerebrospinal fluid, resulting in low scattering and absorption, the sensitivity of NIRS signals in gray matter increases in the reflectance mode, preventing measurement from deep tissues. Therefore, we aimed to investigate the value of NIRS measurement using the transmittance mode in which the source and detection probes are placed on two temporal fossae to allow for the passage of light between them, thus enabling measurement from deep brain tissues. We compared the values obtained using the reflectance and transmittance modes in ELBWIs. We also investigated its efficacy in ELBWIs with patent ductus arteriosus (PDA).

Methods: This single-center observational study was conducted at Saitama Children's Medical Center. The participants included ELBWIs admitted to our center between March 2020 and December 2022. This study was approved by our center's ethics committee. Patient data were extracted from electronic medical records. A three-wavelength (755,

816, and 850 nm) near-infrared time-resolved spectroscopy was used to measure oxyhemoglobin (O_2Hb), deoxyhemoglobin (HHb), total hemoglobin (tHb), tissue oxygen saturation (StO_2), and cerebral blood volume (CBV) levels in reflectance and transmittance modes.
Results: Characteristics of the 39 infants without PDA were as follows: the median gestational age was 25.1 weeks (interquartile range [IQR]: 23.8–23.9), birthweight (mean ± SD: 701±176 g), age at measurement (median: 13 days, IQR: 8–17.5 days), and blood hemoglobin level (mean ± SD: 13.2±2.3 g/dL). Measurement results in reflection and transmittance modes were as follows: O_2Hb (μmol/L), 29.6±8.4 vs. 20.8±6.4 ($p < 0.01$); HHb (μmol/L),17.3±3.1 vs. 15.5±4.0 ($p = 0.03$); tHb (μmol/L), 46.9±9.7 vs. 36.3±9.0 ($p < 0.01$); StO_2 (%), 62.4±6.4 vs. 56.9±7.0 ($p < 0.01$); and CBV (mL/100 g), 2.2±0.4 vs. 1.7±0.4 ($p < 0.01$). Among the seven infants with PDA, results for the respective modes were as follows: O_2Hb (μmol/L), 33.2±12.5 vs. 19.6±5.9 ($p = 0.02$); HHb (μmol/L), 22.6±11.0 vs. 21.4±13.1 ($p = 0.84$); tHb (μmol/L), 55.8±17.4 vs. 41.0±11.0 ($p = 0.06$); StO_2 (%), 59.7±12.9 vs. 50.6±16.8 ($p = 0.21$), and CBV (mL/100 g), 3.0±1.0 vs. 2.2±0.8 ($p = 0.07$).
Conclusion: Among ELBWIs without PDA, all measurement results in transmittance mode were lower than those in reflection mode. This result is believed to reflect the frequently distributed gray matter at the measurement site in reflection mode. In ELBWIs with PDA, the CBV for the respective modes was not significantly different. This result is believed to reflect the fact that the transmittance mode can measure the increase in deep brain tissue due to PDA. Although our study has some limitations, the transmittance mode may be useful for monitoring deep brain tissues.

Keywords: extremely low birth weight infants, patent ductus arteriosus, near-infrared time-resolved spectroscopy, transmittance measurement, reflection measurement

Poster

P7-41

Brain micro-anatomy revealed by 2-photon shadow imaging in vivo

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Getting an accurate, detailed and physiologically relevant view of brain structure and neuronal circuits is a major goal of modern neuroscience. Current large-scale connectomics efforts rely either on EM or MRI, which are either incompatible with live conditions or do not offer cellular resolution. Fluorescence microscopy allows for live imaging with cellular resolution *in vivo*, but has relied on positively labeling of a sparse set of cells, giving an incomplete and biased view of the anatomical organization of brain tissue. Breaking this impasse, super-resolution shadow imaging (SUSHI) established a new paradigm to visualize tissue anatomy in brain slices with nanoscale resolution in an all-encompassing and panoramic way, based on fluorescence labeling of the ACSF and 3D-STED microscopy. Because of the stringent optical demands of super-resolution microscopy, however, the approach has only been applied to living organotypic brain slices so far.
We have now extended the shadow imaging concept to the mouse brain *in vivo*, based on 2-photon shadow imaging (TUSHI) and labeling of the cerebrospinal fluid with a fluorescent membrane-impermeant dye. We present the optical details of the microscope, the labeling strategy for sufficiently bright and homogeneous inverted cellular contrast, as well as the cranial window technique and anesthesia formula for optically clear and mechanically stable access to superficial layers of the cerebral cortex. Despite the diffraction-limited resolution, the new approach opens a stunning window on the micro-anatomical organization of the brain *in vivo*, where cell bodies, dendritic branches of neurons, perivascular spaces (PVS) and spatial heterogeneities in the extracellular space become visible. We reliably estimated PVS in small brain cappilaries and larger blood vessels. We demonstated than size of PVS increases with the size of blood vessels. By adding a second fluorescence channel, the shadow imaging approach reveals the diverse and complex anatomical context of positively labeled neurons, astrocytes, microglia and tumor cells.
In summary, our work demonstrates the feasibility of TUSHI *in vivo* to visualize and quantify brain structure and context with subcellular resolution. It provides a powerful new investigative tool to monitor dynamical changes of brain structures *in vivo* under various (patho-physiological) conditions, such as experience-dependent neuronal plasticity, sleep, aging, stroke, tumor invasion & proliferation.

Keywords: 2 photon imaging, shadow imaging, brain microstructure, perivascular space, extracellular space

P7-42

Mitochondrial calcium uniporter mediates odor learning and memory through neuropeptide release in *C. elegans*

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The known roles of mitochondria in neurons are often limited to their role as ATP suppliers and sources of dangerous ROS. However, recent studies have shown that mitochondria are required for additional, subtle but essential aspects of neuronal function, such as buffering calcium to fine-tune neuronal activity or maintaining firing rate homeostasis in some neurons. In this study, we aimed to investigate whether mitochondrial calcium plays a role in learning and memory by examining mutants that have a defect in the sole route of calcium entry into mitochondria, MCU-1 (mitochondrial channel uniporter). We used model organism *C. elegance* with both aversive olfactory learning and positive associative learning paradigms and found that *mcu-1* mutants were defective in odor learning. The defect was restricted to odors recognized by the AWC^{on} neuron. MCU-1 in neurons or in AWC neurons alone was sufficient to restore learning, suggesting that MCU-1 is required in sensory neurons. Pharmacological inhibition showed that MCU-1 was required at the time of learning. Interestingly, the transgenic strain expressing rescue of MCU by in AWC showed long-term retention of odor memory after only a single training session, in both the aversive learning paradigm and positive associative learning paradigms. This is likely due to the fact that the expression of higher levels of MCU-1 in the transgenic strain was higher than the endogenous levels. This suggests that the amount of MCU-1 in the neuron correlates with learning and memory - a lack of MCU inhibits learning, while too much MCU inhibits forgetting. We noted that similar phenotypes had been observed in the literature in mutants of the neuropeptide NLP-1, its receptor, or in mutants of genes involved in neuropeptide release (1,2). This led us to hypothesize that MCU-1 is required for the control of neuropeptide release in the AWCon neuron. Previous studies show that MCU facilitates neuropeptide release by mediating the production of mitochondrial ROS, which activates protein kinase C (PKC) (3). Consistent with this, we found that treatment with the mitochondrial ROS stressor juglone was sufficient to increase NLP-1 release from AWC. Finally, we found that the *mcu-1* mutant is also defective in salt memory, suggesting that MCU-1 is involved in other forms of learning and memory.

Reference

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Keywords: learning and memory, MCU-1, neuropeptides

P7-43

The increase of exosome secretion after Intracerebral hemorrhage induce delayed onset cognitive impairment

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Over 30% of Intracerebral hemorrhage patients have been suffering for remaining symptoms (specifically, cognitive impairment) after complete remission which called delayed onset injury (DOI). Involvement of neuroinflammation in distant area from brain injury after ICH has been revealed, but how it induces neuroinflammation in distant brain area and underlying mechanism of cognitive impairment after ICH need to be more investigation. To identify the DOI in hippocampal region, we generated the ICH model by collagenase injection to the thalamus and evaluated the neuroinflammation after 7, 14 days. The hippocampal region of ICH mice increases of CD11b and IBA-1 positive cells comparing with vehicle injected mice which represent microglia activation. Consistent with neuroinflammation results, ICH group after 7 days showed significantly decrease memory function comparing with vehicle group by analyzing with barns maze and passive avoidance test. Interestingly, inhibition of exosome secretion by GW4869 attenuated DOI phenotype such as microglial activation and cognitive impairments. Secreted exosomes after brain injury transfer their own information such as intracellular proteins, miRNAs, mRNAs to the adjacent cells and could be contribute to distant brain injury. Because ICH characterized with profound blood brain barrier (BBB) disruption, first, we focused the isolation of exosome from microvessel and performed characterization and optimization of it. After isolation of exosome from brain microvessel, we assessed size and number of particles with nanoparticle tracking analysis (NTA) after isolation of exosome from mouse brain. Comparing with UC (Ultracentrifuge) and UF(Ultrafiltration), isolation by using UC acquired the higher purity of exosomes than UF in brain. Injection of the isolated MV exosomes from ICH mice to the ventricle of wild type mice show the increased CD11b positive cells. Taken together, we suggested that exosomes from damaged MV area could implicate in neuroinflammatory response which can induce DOI with cognitive impairment.

Keywords: exosome, micro vessel, delayed onset brain injury, intracerebral hemorrhage, exosome isolation

P7-44

Examining the involvement of the ventral hippocampus to basal amygdala circuit in shaping contextual fear memories

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The ability to adapt to new surroundings is crucial for survival, as it relies on connecting unfamiliar stimuli with potential threats. Context fear conditioning (CFC) is a commonly used method for exploring the connection between novel stimuli and anticipated stress. The hippocampus, a region known for its role in learning and memory, plays a significant part in the acquisition, encoding, and consolidation processes related to the association between spatial information and fear responses. While the function of the dorsal hippocampus has been extensively elucidated, the function of the ventral hippocampus remains less understood. Previous research has provided evidence about the ventral CA1 (vCA1) region to the basal amygdala (BA) during fear learning. In this study, we focused on identifying engram cells within the vCA1 to BA circuit during CFC. Our findings demonstrated that exposure to a new environment alone is sufficient to increase the activation of neurons in the vCA1 region. However, neurons in the BA region are only activated when mice are exposed to a foot shock associated with the new environment. Furthermore, we are optimizing the use of anisomycin, a protein synthesis inhibitor, to investigate the importance of synaptic strengthening between vCA1 and BA during the fear learning.

Keywords: fear learning, engram, synapse

P7-45

DKK2 regulates adult neurogenesis in hippocampal dentate gyrus by modulating WNT-JNK signaling

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WNT signaling plays a pivotal role in normal brain development and function. Dickkopf-related protein 2 (DKK2), a member of the DKK family (DKK1–4) proteins, modulates WNT signaling either positively or negatively in a tissue-dependent manner through the interaction with WNT co-receptors LRP5/6. However, the role of DKK2 in the central nervous system is unknown. Here we show that DKK2 affects adult neurogenesis in the hippocampus by suppressing WNT signaling. Genetic disruption of DKK2 in mice resulted in enhanced β-catenin expression, JNK phosphorylation, and GSK3β phosphorylation in the hippocampus. Incubation of mouse hippocampal slices with recombinant human DKK2 peptides inhibited Wnt3a- or Wnt5a-mediated β-catenin dephosphorylation and JNK phosphorylation, indicating that DKK2 negatively regulates WNT signaling in the hippocampus. DKK2-mutant mice exhibited fewer newborn neurons in the dentate gyrus subfield and impaired performance during the contextual pattern separation test. These attenuated neurogenesis and impaired pattern separation in DKK2-mutant mice were reversed by chronic inhibition of JNK phosphorylation. Collectively, these results suggest that DKK2 enhances hippocampal neurogenesis through the negative regulation of WNT-JNK signaling.

Keywords: DKK2, adult neurogenesis, pattern separation, contextual fear discrimination, WNT signaling

Poster

P7-46

c-Fos activity in the medulla oblongata of winners and losers in the tube test competition task of rats

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Many sporting events are performed in competition with others. In previous studies using a tube test in which mice or rats were pushed against each other from both sides of a single tube, activation of the medial prefrontal cortex (mPFC) was found to be involved in winning or losing a competition related to social rank. Not only are motor control behaviors like "pushing back" or "running away" crucial during the competitive phase of the tube test, but also the blood flow supply to the active muscles that generate the motor action However, the descending pathway from the mPFC to the brain regions involving autonomic regulation of blood pressure control, such as the hypothalamus, amygdala, and medulla oblongata, are unknown. The mPFC has strong anatomical connections with the amygdala and other regions related to the regulation of autonomic responses such as emotion and circulatory regulation. Therefore, we hypothesized that winners and losers exhibit different autonomic-related responses during social competition, and such differences in response are due to different brain neural networks. To verify this hypothesis, we used the tube test to assess behavior during social competition and performed an immunohistochemical analysis of the brains of winners and losers. For the behavioral evaluation of the tube test, Wistar rats (n = 24, 8 weeks old or older) were placed one on each side of a 5.5 cm diameter tube with a partition inside When two rats were pitted against each other, the rat that pushed the opponent out was defined as the "winner", and the rat that was pushed out was defined as the "loser". Four rats were used for each experiment, and the rats played a total of 12 matches per day in a round-robin fashion, with different combinations of matches. The tube test was performed until the social ranks were fixed (i.e., the results of the games between each rat were fixed for 2 to 3 consecutive days). Ninety to 120 min after the end of the experiment on the last day, brain tissues were extracted and examined for c-Fos immediate early gene expression in brain regions between social ranks. The results of the behavioral tube competition test showed that the winners were not stable in the early stages (about a few days), but in the later stages (about 2 weeks), the winners were stable. Regarding c-Fos activity, a predominant activation was observed in the mPFC as in previous studies. Interestingly, a trend toward higher activation of the dorsal medulla oblongata was observed in the lower-ranked, than higher-ranked animals. These results suggest that not only the frontal cortex, such as the mPFC but also medullary regions important for autonomic control may be involved in the process of social competition formation.

Keywords: tube test, competition, rat, medulla, c-Fos

P7-47

Effects of curcumin and gamma-oryzanol solid dispersion on learning and memory of middle-aged rats

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Memory loss begins in the middle-aged, the prevention of the cognitive impairment before aging is essential. Oxidative stress causes molecular alteration, structural damage, and brain dysfunction, which associates with cognitive impairment. Safeguarding against brain deterioration becomes essential, emphasizing the need for proactive prevention. Numerous studies have consistently highlighted the remarkable antioxidant and anti-inflammatory properties of curcumin and γ-oryzanol. However, curcumin and γ-oryzanol had a low aqueous solubility. To overcome this hurdle and maximize their solubility and stability, a solid dispersion technique was employed in the preparation of curcumin and γ-oryzanol. This study aims to evaluate the effects and mechanisms of γ-oryzanol solid dispersion (GOSD) and curcumin solid dispersion (CURSD) on learning and memory in six different groups of male rats (n=5/group). The initial group comprises adult controls consisting of 6-week-old male rats, while the remaining five groups consist of 42-week (middle-aged) male rats. These groups are designated as the control group, the GO group (GOSD 10 mg/kg·BW), the Cur group (CURSD 50 mg/kg·BW), the GO-LCur group (GOSD 10 mg/kg·BW plus CURSD 25 mg/kg·BW), and the GO-HCur group (GOSD 10 mg/kg·BW plus CURSD 50 mg/kg·BW). The substances are administered once daily via oral gavage for 42 consecutive days. The GO-HCur group demonstrated a noteworthy enhancement in learning and memory performance, as evidenced by their remarkable performance in both the Morris water maze and the spontaneous tendency novel object test. Furthermore, GO-HCur significantly decreased the levels of lipid peroxidation, increased superoxide dismutase and catalase activity. This improvement coincided with elevated levels of c-Fos and NMDAR1 expression observed in both the hippocampus and prefrontal cortex. The findings revealed that the administration of GOSD at a dosage of 10 mg/kg combined with CURSD at a dosage of 50 mg/kg effectively enhanced learning and memory performance in the middle-aged rats.

Keywords: curcumin, γ-oryzanol, memory, antioxidant, NMDAR1

P7-48

Postnatal development of hippocampal function: Contextual learning performance correlates with pathway-specific plasticity at hippocampal CA1 synapses

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Synaptic plasticity in the dorsal hippocampal CA1 region plays an important role in learning and memory. Understanding developmental changes in the hippocampal function, together with the pathway-specific synaptic plasticity, is essential for elucidating the mechanisms underlying cognitive development. By blocking synaptic delivery of AMPA receptor into the CA1 synapses, we have demonstrated the causal link between synaptic plasticity and contextual learning (Mitsushima et

al., PNAS 2011, Nat Commun 2013). Furthermore, using multiple hippocampal dependent learning tasks, we demonstrated a task-dependent critical period of hippocampal learning in juvenile rats (Sakimoto et al., Sci Rep 2022). For the inhibitory avoidance (IA) task, juvenile rats failed to establish learning, but their performance clearly improved by postnatal day 21 (PN21), indicating a critical period for contextual learning between PN16 and PN21 of age.
In this study, we examined developmental changes in pathway-specific plasticity at dorsal CA1 synapses after IA training. Male rats were subjected to the IA task, and acute hippocampal slices were prepared after the retrieval test for whole-cell patch clamp analysis. Evoked excitatory postsynaptic currents (EPSCs) were analyzed by stimulating ECIII-CA1 or CA3-CA1 input fibers, and the data were compared to the untrained control group. At the CA3-CA1 synapses, IA training consistently increased AMPA/NMDA ratios from PN16 to 23. In contrast, at the ECIII-CA1 synapses, the training increased AMPA/NMDA ratio from PN 16 to 21, but not PN 22 and 23. Although neither CA3-CA1 and ECIII-CA1 synapses showed significant correlation between the AMPA/NMDA ratio and the learning performance, both synapses showed a positive correlation after PN23 of age, suggesting that there is a critical period between AMPA receptor-mediated synaptic plasticity and the learning performance.
IA training diversifies synapses by strengthening not only excitatory synapses but also inhibitory synapses. In this study, we analyze miniature EPSCs and IPSCs of CA1 synapses along with learning performance to further capture developmental changes. Multiple regression analysis of learning performance and the amplitude and frequency of inhibitory and excitatory synaptic currents in individuals will reveal a critical period of synaptic plasticity linked to the learning performance.

Keywords: developmental change, synaptic plasticity, AMPA receptor, critical period, GABAA receptor

P7-49

Finding clinical indicator for evaluating sleep quality of non-invasive core temperature changes with polysomnography

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Telemedicine's potential and importance has recently been highlighted due to the COVID-19 pandemic, which stretched the limits of in-person medical care. However, sleep disorders have fallen behind in the telemedicine sector due to the diagnostic dependence on in-hospital polysomnography (PSG) studies. This study proposed core body temperature (CBT) as a non-invasive, telemedicine-friendly, cost-effective diagnostic indicator for sleep disorders. Thermoregulatory behavior has been shown to reinforce the circadian rhythm, demonstrating the value of CBT as an effective biomarker for evaluating sleep parameters. The aim of this study was to determine the relationship between CBT and sleep stage and demonstrate its accuracy in assessing sleep efficiency and sleep disorders. Clinical and observational studies were performed on 12 adult subjects, as well as a PSG using NOX A1, with simultaneous verification of CBT change using a 3MTM Bair Hugger sensor. A survey was conducted before and after sleep, which was monitored from 22:00 to 06:00 for 8 hours. The Graphpad Prism program was used to analyze CBT change patterns in 30 second intervals according to sleep stage, ranging from awake, to REM, to NREM stages 1 through 3, and in correlation with sleep quality, measured primarily through the Pittsburgh Sleep Quality Index (PSQI). Data was obtained on change in core body temperature, sleep, cardiac, respiratory, and oximetry summaries, snore data, limb movements, and body position in respiratory events. Using noninvasive measurements of CBT in conjunction with polysomnography, 4 subjects were diagnosed with mild obstructive sleep apnea-hypopnea syndrome (OSAHS), 1 subject with moderate OSAHS, 3 subjects with severe OSAHS, 3 subjects with mild/moderate/severe OSAHS, and 1 subject was not diagnosed with a sleep disorder. Through graphical analyses of 12 subjects, it was confirmed that CBT decreases as sleep progresses to deeper sleep stages and increases when arousal occurs. The results of this study clearly indicate a correlation between CBT and sleep stage, and its diagnostic potential for sleep disorders. Therefore, it is proposed that CBT may be used as a telemedicine-friendly clinical index for sleep disorder patients while being attuned to a pandemic-conscious, patient-centered healthcare system.

Keywords: core temperature, sleep quality, polysomnography, sleep disturbance, thermoregulatory behavior

P7-50

Immunotherapy targeting plasma ASM is protective in a mouse model of alzheimer's disease

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Acid sphingomyelinase (ASM) has been implicated in neurodegenerative disease pathology, including Alzheimer's disease (AD). However, the specific role of plasma ASM in promoting these pathologies is poorly understood. Herein, we explore plasma ASM as a circulating factor that accelerates neuropathological features in AD by exposing young APP/PS1 mice to the blood of mice overexpressing ASM, through parabiotic surgery. Elevated plasma ASM was found to enhance several neuropathological features in the young APP/PS1 mice by mediating the differentiation of blood-derived, pathogenic Th17 cells. Antibody-based immunotherapy targeting plasma ASM showed efficient inhibition of ASM activity in the blood of APP/PS1 mice and, interestingly, led to prophylactic effects on neuropathological features by suppressing pathogenic Th17 cells. Our data reveals new insights into the potential pathogenic mechanisms underlying AD and highlights ASM-targeting immunotherapy as a potential strategy for further investigation.

Keywords: Alzheimer's disease, plasma ASM, Parabiosis, Th17 cells, Antibody-based immunotherapy

P7-51

Discovery of a novel dual-action small molecule that improves multiple Alzheimer's disease pathologies

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Alzheimer's disease (AD) is characterized by complex, multifactorial neuropathology, suggesting that small molecules targeting multiple

neuropathological factors are likely required to successfully impact clinical progression. Acid sphingomyelinase (ASM) activation has been recognized as an important contributor to these neuropathological features in AD, leading to the concept of using ASM inhibitors for the treatment of this disorder. Here we report the identification of KARI 201, a direct ASM inhibitor evaluated for AD treatment. KARI 201 exhibits highly selective inhibition effects on ASM, with excellent pharmacokinetic properties, especially with regard to brain distribution. Unexpectedly, we found another role of KARI 201 as a ghrelin receptor agonist, which also has therapeutic potential for AD treatment. This dual role of KARI 201 in neurons efficiently rescued neuropathological features in AD mice, including amyloid beta deposition, autophagy dysfunction, neuroinflammation, synaptic loss, and decreased hippocampal neurogenesis and synaptic plasticity, leading to an improvement in memory function. Our data highlight the possibility of potential clinical application of KARI 201 as an innovative and multifaceted drug for AD treatment.

Keywords: Alzheimer's disease, ASM direct inhibitor, GHSR1 alpha agonist, memory improvement, small compound

Poster

P7-52

UPRmt mediates anti-inflammatory response for attenuation of kaolin-induced hydrocephalus

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Ventriculomegaly induced by the abnormal accumulation of cerebrospinal fluid (CSF) leads to hydrocephalus, which is accompanied by neuroinflammation and mitochondrial oxidative stress. The mitochondrial stress activates mitochondrial unfolded protein response (UPRmt), which is essential for mitochondrial protein homeostasis. However, the association of inflammatory response and UPRmt in the pathogenesis of hydrocephalus is still unclear. To assess their relevance in the pathogenesis of hydrocephalus, we established a kaolin-induced hydrocephalus model in 8-week-old male C57BL/6J mice and evaluated it over time. We found that kaolin-injected mice showed prominent ventricular dilation, motor behavior defects at the 3-day, followed by the activation of microglia and UPRmt in the motor cortex at the 5-day. In addition, PARP-1/NF-κB signaling and apoptotic cell death appeared at the 5-day. By the silencing of Atf5 in activated microglia which is an upstream molecule of UPRmt, we identify the production of pro-inflammatory cytokines interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α), but also decreased mitochondrial membrane potential (MMP). Our results suggest that ATF5-dependent UPRmt in the microglia acts as a protective mechanism during neuroinflammation, and may be a potential treatment target for reducing neuroinflammation, it provides a new sight for the pathogenic target of hydrocephalus.

Keywords: hydrocephalus, UPRmt, ATF5

P7-53

Amyloid β-induced upregulation of TREK channels mediate congnitive deficits

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Understanding early functional changes in Alzheimer's disease (AD) is crucial for developing effective treatments. Modulation of cellular electrical activity through ion channels, including TREK (TREK-1 and TREK-2) K_{2P} channels, has been implicated in AD pathology. However, the involvement of TREK channels in early AD pathology remains unreported. In this study, we utilized an $AD_{Aβ1-42}$ mouse model induced by stereotaxic injecting oligomeric amyloid beta protein ($Aβ_{1-42}$) into the hippocampus to investigate the initial pathological response at 3 days post injection. The $AD_{Aβ1-42}$ mouse exhibited AD-like pathology, including spatial memory decline, depression-like behavior, increased glial activity, and elevated level of oxidative stress and inflammation. Interestingly, the expression of TREK-1 and TREK-2 channels was significantly upregulated in the hippocampal CA1 region of $AD_{Aβ1-42}$ mice, approximately 2-fold higher compared to wild-type mice. However, in TREK-1 and TREK-2 deficient ($TREK^{-/-}$) mice, the $Aβ_{1-42}$-induced AD-like pathology did not show an increase and remained similar to the control group. Furthermore, the $TREK^{-/-}$ mice exhibited rescue from upregulated inflammatory chemokine release, lack of MAP2 or BDNF expression, and increased cell death observed in $AD_{Aβ1-42}$ mice. These findings indicate that the upregulation of TREK channels is involved in early AD pathology. Moreover, their role in regulating cognitive function suggests their potential as a therapeutic target for AD treatment.

Keywords: Alzheimer's disease, TREK-1, TREK-2, neuron, glia

P7-54

Neuroprotective effect of neuropeptide-w against hypoxic-ischemic brain damage in newborn rats

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Background: Hypoxic-ischemic encephalopathy (HIE), an important cause of acute neonatal brain injury, has no preventive or curative treatments yet. Neuropeptide W (NPW), isolated from hypothalamus, has analgesic and antiepileptic effects. We aimed to investigate possible neuroprotective effects of NPW on brain damage of newborn rats induced with HIE.

Methods: Postnatal 7-day-old Sprague-Dawley pups (n=40) were divided into 4 HIE groups and a sham-surgery (control) group. Following ligation of right common carotid arteries under anesthesia, pups were placed in an incubator (37°C) aerated with hypoxic gas mixture (92% nitrogen+8% oxygen) for 120 min to induce HIE, while sham-surgery group was kept with mothers. HIE-induced neonates were treated with saline or NPW (0.1, 1 or 10 μg/kg/day) for 3 days. The pups were decapitated at 24 hours after last treatment and brain tissue levels of

antioxidant glutathione (GSH), luminol, lucigenin and malondialdehyde (MDA), myeloperoxidase (MPO) enzyme activity were measured. Neuronal damage, quantification of GFAP- and TUNEL-positive cells were determined by histopathological analyses. Statistical comparisons were performed by ANOVA and Student's t-tests.
Results: Compared to control group, MDA ($p<0.05$) and MPO ($p<0.001$) levels were significantly increased in saline-treated HIE group, while MDA levels and MPO activity were decreased in NPW-treated HIE groups ($p<0.01$). While antioxidant GSH content in saline-treated HIE group was not different from control group, GSH levels were increased in all NPW-treated HIE groups ($p<0.01$). Luminol and lucigenin levels, showing radical formation, were increased by HIE ($p<0.001$), but decreased in NPW-treated HIE groups ($p<0.01$). Compared with control, neuronal damage score of cortex, hippocampal dentate gyrus and CA3 brain regions in saline-treated HIE group were increased ($p<0.001$) and these damage scores were reduced with NPW treatment in all 3 brain regions ($p<0.05$-0.001). Elevated scores of GFAP- and TUNEL-positive cells in all studied brain regions of saline-treated HIE group ($p<0.05$-0.001) were depressed in NPW-treated HIE groups ($p<0.05$-0.001).
Conclusion: NPW effectively increased antioxidant level, inhibited lipid peroxidation, neuronal injury and neutrophil infiltration in brain tissues of newborn rats with HIE, suggesting a potential neuroprotective action of NPW in acute hypoxia-ischemic injury of newborns.

Keywords: neuropeptide W, Hypoxia-ischemia, oxidative damage, brain, newborn

P7-55

Released glutamate through Best1 induces invasion, migration and low cell density of GBM

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Glioblastoma (GBM) is a highly aggressive brain tumor characterized by various symptoms such as headaches, vomiting, blurred vision, seizures, and a poor prognosis with a median survival of 10 to 15 months. Current treatment strategies for GBM include radiation therapy, chemotherapy, and surgical removal of the tumors. However, complete surgical resection is challenging due to the invasive nature of GBM cells, which migrate and infiltrate surrounding brain tissue. During this process, GBM cells interact with different cell types, including astrocytes, neurons, and endothelial cells. One important mechanism of interaction involves the release of gliotransmitters, with intracellular Ca^{2+} increase playing a significant role in invasion. Here, we investigated the impact of glutamate, a major gliotransmitter released in response to intracellular Ca^{2+} increase. We focused on the role of Best1 (Bestrophin 1), a Ca^{2+}-activated anion channel activated by G_qPCR (G_q protein-coupled receptor) activation, in mediating the release of glutamate by GBM cells. By blocking the release of glutamate, we observed a decrease in the motility and invasive potential of GBM cells in vitro. Additionally, we conducted experiments both in vitro and in vivo, which demonstrated that reducing the expression of Best1 resulted in a decrease in cell density.

Keywords: glioblastoma, glutamate, Bestrophin1, GPCR

P7-56

Glutamate via system xc- induces invasion in glioblastoma

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Glioblastoma(GBM) is the most common and lethal brain tumor with particularly high invasive capacity. There are a number of factors that determine invasion in GBM, including Ca^{2+} and glutamate. Glutamate is exported by cysteine glutamate antiporter, *SLC7A11* or system x_c^- (xCT), which is inhibited by Sulfasalazine(SAS). Previous studies have reported that invasion was inhibited by SAS, and we used SAS to determine the effect of reduced glutamate levels on $Ca2^+$ in GBM. Our study shows that secreted glutamate level and invasion capacity were reduced by SAS. We anticipated that inhibiting xCT, as we did with the SAS treatment, would have a similar or even more dramatic effect, so we made shRNA of xCT and performed the same experiment. We expect that inhibition of glutamate through xCT knockdown in GBM will reduce the invasive nature of GBM and that these studies will have similar effects while addressing the negative aspects of SAS from a clinical perspective.

Keywords: glioblastoma, system xC-, glutamate, invasion

P7-57

A novel NMDA receptor modulator rises a new hope for the treatment of multiple system atrophy: From preclinical models to patients

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NMDA receptor (NMDAR) is an essential glutamate receptor and as a contributor to neurodegenerative disorder. Until now, NMDAR (especially glycine modulatory site (GMS) of the NMDARs) has been considered and proposed to be a potential therapeutic target for the treatment of neurological disorders, such as multiple system atrophy (MSA). MSA, an atypical parkinsonism, is a fatal and rapidly progressive neurodegenerative disease with autonomic dysfunction and cerebellar deficits. Up to now, the precise mechanism underlying pathogenesis of MSA remains unclear. Unfortunately, currently available medications have not been proven efficacious and reliable in treating symptoms of MSA. The need to develop novel therapeutic agents for the "unmet medical needs" in patients with MSA is of upmost importance. Take advantage of virtual high-throughput screening and AI-based ADMET predicted assay, a novel NMDAR modulator, RS-D7, was discovered with good safety and efficacy profile. It is urgent and imperative to evaluate the therapeutic potentials of RS-D7 in MSA from basics to clinical trials. To elucidate the effect of RS-D7 on MSA in drug development, a set of experiments was conducted. First, RS-D7 acted as a potent D-amino acid oxidase (DAO) inhibitor (IC_{50} = 0.32 uM) with direct competitive inhibition. Then, using single particle tracking with NR1 subunit of NMDAR in primary culture, the surface dynamics of hippocampal NMDARs were decreased by MK-801 (dizocilpine, a NMDAR antagonist) but significantly alleviated by RS-D7. Next, *in vivo* animal experiments were performed with RS-D7 treatment using pharmacological and genetic mouse models of MSA. RS-D7 demonstrated a high safety profile on wild-type (WT) mice. All ataxic behaviors and NMDAR-mediated fEPSPs deficits observed in MK-801-induced mouse model were alleviated after RS-D7 treatment.

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Moreover, MSA-related behavioral deficits, reduction of membrane-bound NMDAR subunits, and abnormal inflammatory responses in the cerebellum of TgM83 mice overexpressing human A53T α-synuclein were also reversed by RS-D7. Final, an open-labeled, investigator-initiated proof-of-concept clinical trial in MSA patients was conducted with RS-D7pro, which is a market drug and a prodrug of RS-D7. The clinical rating results indicated that three ataxic scores (SARA, UMSARS and ICARS) were significantly improved in MSA patients after 12-week RS-D7pro treatment. Furthermore, the symptoms of MSA rebounded and worsened 12 weeks after discontinuing RS-D7pro. Collectively, these solid evidence and promising results support the therapeutic potential of RS-D7 in MSA and offer new hope for MSA patients.

Keywords: NMDA receptor, D-amino acid oxidase inhibitor (DAOI), multiple system atrophy (MSA), translational research, drug development

P7-58

Decipher the role of aryl hydrocarbon receptors in aromatic-uremic toxins induced cognitive disorder

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Chronic kidney disease (CKD) is with a characterization of progressing loss of renal function; commonly, CKD progression leads to multiple comorbidities, cognitive dysfunction is one of them. Our previous study reported that accumulation of uremic toxins, especially indoxyl sulfate (IS) and p-cresol sulfate (PCS), could penetrate into brain tissue and induce neuronal inflammation and contribute to CKD-triggered cognitive impairment. The current study aims to understand how IS and PCS passing through endothelial cells and affecting endothelial physiology, consequently, compromises cerebral-endothelial and blood-brain-barrier (BBB) function. The results of Western blotting assay indicated expression of inflammatory cytokines, caspase-1, IL-1β, and IL-18, and tight-junction proteins were altered in IS/PCS-treated cerebral endothelial cells. In the meantime, immunocytochemistry images showed that the aryl hydrocarbon receptor (AhR) was activated and relocated to nuclei and the organic anion transporter (OAT) inhibiter, chloroquine, prohibited IS-induced AhR nuclear relocation of endothelial cells. The mitochondrial function was examined by Agilent Seahorse XF analyzer, which indicated treatment of IS and PCS compromised ATP production of mitochondria in cerebral-endothelial cells respectively. Moreover, the ChIP-seq assay was performed to determine genes that are regulated by AhR. The analytical results of ChIP-seq suggested AhR-regulated genes involving in function of cell junction, cell adhesion, integral component of membrane, organelle envelope, cytoplasmic vesicle, intracellular transport, and G-protein signaling. Therefore, we postulated that IS and PCS pass through endothelial membrane via certain types of OATs/OATPs and activate AhRs relocating to nuclei, subsequently, induce endothelial inflammation, alter expression of tight-junction proteins, and dysregulate permeability of BBB. The goal of our study is to decipher mechanisms of aromatic uremic toxins, specially IS and PCS, induced cognitive impairment and find out possible treatments via blocking IS/PCS endothelial transcytosis, suppressing endothelial AhR activity, preventing endothelial dysfunction, and maintaining BBB permeability.

Keywords: chronic kidney disease, cognitive impairment, Aryl hydrocarbon receptor, Indoxyl sulfate, organic anion transporter

P7-59

The interplay between olfactory impairment and cognitive decline in an Alzheimer's disease mouse model

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Alzheimer's disease (AD) is a neurodegenerative disease characterized by cognitive decline. Emerging studies revealed that many AD patients have clinical symptoms of olfactory dysfunction, and impaired olfaction is associated with cognitive decline. In addition, hyposmia and anosmia showed a higher rate of suffering from AD, but the link between olfactory decline and cognitive deficits in AD remains unclear. A widely used AD research mouse model, 3xTg-AD mice, was utilized to test olfaction and cognitive functions at different ages. Our results showed that 9-month-old 3xTg-AD mice had both olfactory and cognitive impairments, and there indeed had a correlation between olfactory and cognition. To further investigate whether altered olfactory bulb activity affects cognitive function, we injected excitatory DREADDs (AAV5-hSyn-hM3D(Gq)-mCherry) virus to activate the olfactory neurons in 8-month-old 3xTg-AD mice and observed cognitive performances. Next, we will decode neural circuits from olfactory nerves to cognition-related brain regions and explore whether abnormal inflammatory microenvironment factors are involved in the underlying mechanisms. The results of this study will open new avenues of AD research and provide new insights into the association between olfaction and cognitive function in AD.

Keywords: Alzheimer's disease, cognition, olfaction, neural circuit, neuroinflammation

P7-60

The potential therapeutic effects of cannabidiol encapsulated lipid-nanoparticles for Parkinson's disease with diabetes in a rat model with cognitive impairment

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Memory impairment is a prominent neurological manifestation in individuals with diabetes and Parkinson's disease. Cannabidiol (CBD), the non-psychoactive component of the cannabis plant, has demonstrated potential neuroprotective and memory-enhancing effects. However, the limited solubility and bioavailability of CBD present challenges in achieving optimal therapeutic outcomes. This study aimed to compare the efficacy between CBD lipid nanoparticles and CBD in improving memory in a rat model of diabetes with Parkinson's disease.
Twenty-four adult male Wistar rats were divided into three groups (eight rats per group): diabetic Parkinsonian (DP) rats treated with vehicles (3 mg/mL sunflower oil), DP rats treated with CBD, and DP rats treated with CBD lipid nanoparticles. Parkinsonism was induced by intraperitoneal injections of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), while diabetes was induced by a single injection of streptozotocin (35 mg/kg) and a high-fat diet. The treatment groups received daily oral

doses of CBD or CBD lipid nanoparticles (20 mg/kg) for a duration of four weeks. Memory performance was assessed using the Morris water maze (MMW) test.
The results demonstrated that both CBD and CBD lipid nanoparticles significantly improved memory performance in DP rats compared to the untreated DP group. However, the CBD lipid nanoparticles demonstrated superior efficacy in memory enhancement, as demonstrated by decreased latency in the learning task and latency to reach the target platform in the MWM test. Furthermore, hippocampal histology analysis showed no significant changes, indicating the neuroprotective effects of both CBD formulations with reduced toxicity and non-neuronal cell death. Additionally, biochemical assays indicated that CBD lipid nanoparticles restored dopamine levels in the striatum tissues of the DP rats.
In conclusion, both CBD and CBD lipid nanoparticles exhibited beneficial effects in improving memory function in diabetic rats with MPTP-induced Parkinsonism. However, CBD lipid nanoparticles demonstrated enhanced therapeutic efficacy, surpassing CBD alone, and offering prolonged memory enhancement. These findings underscore the potential of utilizing lipid nanoparticle formulations to augment the delivery and efficacy of CBD in the treatment of memory impairment associated with diabetes and Parkinson's disease. Further investigations are necessary to elucidate the underlying mechanisms and optimize the formulation parameters for potential clinical translation.

Keywords: cannabidiol, diabetes, lipid nanoparticles, memory, Parkinson's disease

P7-61

Naringin enhances long-term potentiation and recovers learning and memory deficits of amyloid-beta induced alzheimer's disease-like behavioral rat model

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Naringin (4', 5, 7-trihydroxy flavanone 7-rhamnoglucoside) is a major constituent of flavanone glycoside from grapefruits and related citrus species such as *Citrus paradise*, *Citrus sinensis*, *Citrus unshiu*, and *Artemisia selengensis*. Previous studies have suggested that diverse biological and pharmacological activities of naringin possess ardioprotective, anti-inflammation, anti-oxidative, and anti-apoptotic properties. However, the effect of naringin on Alzheimer's disease (AD) caused by amyloid beta (Aβ) has not been clearly studied, and there are few studies on the electrophysiological aspect. We aimed to investigate the electrophysiological effect of naringin on AD in the hippocampus. In addition, animal behavior tests and western blot analysis were added to reveal that naringin could be a potential treatment for AD.

Keywords: Naringin, Amyloid beta, Alzheimer's disease, long-term potentiation, synaptic

P7-62

Potential effects of short-term mindful awareness practice in improving neuroprotection among individuals with mild cognitive impairment

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Mild cognitive impairment (MCI) represents the intermediate stage of a continuum ranging from normal cognitive functioning to neurodegenerative diseases. Currently, the lack of its specific treatment opens potential non-pharmacological therapeutic approaches to modify or slow the disease progress. This study investigated the effects of mindful awareness practice on the kynurenine pathway (KP) related neuroinflammatory markers in the development of cognitive decline at initial stage of dementing disease process. MCI individuals with a Monteral Cognitive Assessment test (MOCA) score of 18-25 attending the memory clinics at Hospital Tuanku Jaafar, Seremban, Malaysia were randomized into 2 cohorts: MCI participants (n=17, mean age of 65.8 ± 7.9 years) undertaking mindful awareness practice (a guided focus breathing and body scan, 30-minute daily for 21 days), and those (n=19, mean age of 68.6 ± 11.3 years) with familiar support who served as control. Mindful awareness practice significantly increased serum concentrations of KP inducer, interferon-gamma (IFN-g), ($p<0.05$) while significantly decreasing concentrations of tryptophan (TRP) ($p<0.05$), and kynurenine ($p<0.05$). Although not significant, higher concentrations of neuroprotectant picolinic acid (PIC), and lower concentrations of neurotoxic metabolites, 3-hydroxykynurenine (3-HK) and quinolinic acid (QUIN) were observed after practice. No significant changes were noted for other studied KP modifying factors; total depression and anxiety stress scores, serum concentrations of cortisol, and brain-derived neurotrophic factor (BDNF). As for MCI participants with familiar support, concentrations of cortisol ($p<0.01$) and IFN-g ($p<0.001$) were higher while TRP ($p<0.05$) were lower at 21 days. However, no significant changes in KP metabolites were noted over the 21 days. As neurotoxin 3-HK and QUIN are crucial in developing neuroinflammation and thus neurodegeneration, the resulted decreasing trend in these kynurenines together with increased PIC indicates the possible neuroprotective role of mindful awareness practice in MCI. Larger sample size studies with longer duration of interventions are recommended for future research.

Keywords: mild cognitive impairment, mindful awareness practice, brain-derived neurotrophic factor, cortisol, kynurenine metabolites

Poster

P7-63

The impact of dapoxetine on pilocarpine-induced status epilepticus

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Status epilepticus (SE) is a prolonged seizure activity that affects individuals of all age groups and is associated with a mortality rate of approximately 20%. It is characterized by continuous seizure symptoms lasting for an extended period and can be triggered by various factors, including brain trauma, tumors and vascular diseases. SE leads to excess glial cell activation, neuronal cell death, and disruption of neurotransmitter balance.
In this study, we aimed to investigate the effects of dapoxetine on SE using a well-established animal model of human temporal lobe epilepsy induced by pilocarpine. Dapoxetine, a selective serotonin reuptake inhibitor (SSRI), was administered intraperitoneally at a dose of 4 mg/kg one hour prior to pilocarpine injection. Dapoxetine significantly delayed the onset time of the first seizure. To assess neurotoxicity, tissue samples were collected three days after SE induction and subjected to Fluoro-Jade B and Cresyl violet staining. These analyses demonstrated that dapoxetine treatment effectively reduced neuronal cell death in the hippocampal CA1 region compared to the group treated with pilocarpine alone. Furthermore, immunofluorescence analysis was performed to evaluate the impact of dapoxetine on SE-induced glial activation. It was observed that dapoxetine attenuated the expression of CD11b, a marker for activated microglia. Considering the involvement of glutamate receptors in seizure activity and neuronal death, we investigated the effect of dapoxetine on the expression levels of glutamate receptor 1, 2 (GluR1, 2), and metabotropic glutamate receptor 5 (mGuR5) using immunofluorescence and western blot analysis. Our results indicated that pilocarpine decreased the expression of GluR1, 2 and mGluR5 compared to the sham group, whereas dapoxetine treatment increased their expression levels.
In summary, our study provides compelling evidence that dapoxetine effectively delays the onset of seizures and reduces seizure susceptibility in pilocarpine-induced SE. Moreover, dapoxetine exhibits notable neuroprotective properties by mitigating SE-induced neuronal cell death and neuroinflammation. Additionally, dapoxetine interferes with the downregulation of GluR1, 2 and mGluR5 expression induced by SE. These findings highlight the potential of dapoxetine as a promising therapeutic approach for the treatment of SE.

Keywords: status epilepticus, dapoxetine, neuronal death, glial activation, glutamate receptor

P7-64

The effect of obesity on pilocarpine-induced status epilepticus

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Obesity, characterized by a body mass index (BMI) of 30 or more, is a rapidly growing global health concern associated with various diseases such as cancer, diabetes and an increased risk of neurodegenerative disorders. Status epilepticus (SE) refers to a condition characterized by prolonged seizures lasting over 5 minutes or occurring more than twice within a 5-minute period. Although obesity is recognized as a comorbidity in epilepsy, comprehensive studies exploring the correlation between obesity and epilepsy are still lacking.
To investigate the effects of obesity on status epilepticus, we employed a well-established leptin-deficient ob/ob mice model and induced status epilepticus using pilocarpine, a widely recognized animal model for human temporal lobe epilepsy. Intraperitoneal administration of pilocarpine to normal C57BL/6J (+/+) mice and obese leptin-deficient ob/ob (C57BL/6J -/-) mice resulted in a significantly faster onset of the first seizure and an accelerated progression towards status epilepticus in the ob/ob mice. Moreover, Fluoro Jade B staining revealed a substantial increase in neuronal apoptosis in the CA1 and hilus regions of the hippocampus in ob/ob mice compared to pilocarpine-treated normal mice. Immunofluorescent staining was employed to assess glial cell hyperactivity induced by SE, demonstrating elevated expression of glial fibrillary acidic protein (GFAP), a marker of glial cells, in ob/ob mice. Western blot analysis further confirmed a significant increase in GFAP expression in ob/ob mice. Additionally, the neuroinflammatory response triggered by excessive glial cell activation was evident through the upregulation of proinflammatory cytokines interleukin 1 beta and tumor necrosis factor-α in ob/ob mice. Enhanced expression of lipocalin 2 (LCN) and phosphorylated signal transducer and activator of transcription 3 (p-STAT3), both associated with amplified neuroinflammation, was also observed in ob/ob mice. Notably, necroptosis, a mechanism of neuronal cell death, involving the mixed lineage kinase domain like protein (MLKL) as a signaling mediator, exhibited significantly increased expression of phosphorylated MLKL (p-MLKL) in ob/ob mice.
Collectively, our findings suggest that obesity enhances seizure susceptibility, upregulates GFAP expression through LCN2 and STAT3 phosphorylation, and promotes necroptosis via exacerbated neuroinflammation, ultimately resulting in increased neuronal death in obese individuals. This study provides foundational insights into the mechanisms through which obesity influences acute seizure and contributes to the existing knowledge base for further research on the interplay between obesity and seizure.

Keywords: obesity, status epilepticus, hippocampal cell death, inflammation, necroptosis

P7-65

Effects of near infrared laser therapy in an In vitro model of alzheimer's disease

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Alzheimer's disease is a prevalent neurodegenerative disorder primarily affecting the elderly, and currently, there is no cure available. This condition is known to be mainly associated with an increase in Amyloid beta (Aβ) protein. Recent research has been exploring the potential effects of photobiomodulation using red or near-infrared light on Aβ-induced neurodegeneration. However, the cellular mechanisms by which photobiomodulation regulates neurodegeneration are still in the early stages of investigation. In this study, we aimed to investigate the modulatory effects and underlying mechanisms of photobiomodulation on cell toxicity and synaptic degeneration induced by Aβ 1-42, the principal causative substance known for Alzheimer's disease.
We examined the effects of near-infrared laser diode (LD) at 808 nm on the cellular toxicity of Aβ 1-42 in primary cultured cortical cells. Through analysis of cell viability using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, we observed significant effects from LD laser irradiation using a power of 10mW and energy of 30J. Exposure to Aβ 1-42 led to a significant decrease in the number of post-synaptic density protein-95 (PSD95) puncta, representative of synaptic

contacts between cortical neurons, along with accompanying morphological changes indicating synapse loss. However, this effect was attenuated by near-infrared LD laser therapy. Additionally, we observed a reduction in the aggregation of amyloid peptides and phosphorylated tau protein, both hallmark features of Alzheimer's disease, following near-infrared laser irradiation. Moreover, in both isolated cortical astrocytes and microglia, the increased intensity of Glial Fibrillary Acid Protein (GFAP) and IBA-1 caused by Aβ 1-42 exposure was significantly decreased by near-infrared laser irradiation. Notably, near-infrared laser irradiation significantly suppressed the phosphorylation of Mixed Lineage Kinase domain-Like protein (MLKL), which is associated with neurodegeneration caused by Aβ 1-42-induced necroptosis.
These findings suggest that near-infrared LD laser therapy plays a crucial role in suppressing glial cell activation and neuronal necroptosis induced by Aβ 1-42, thereby effectively alleviating neurodegeneration. Consequently, this laser therapy holds great promise as a significant treatment approach for delaying or treating Alzheimer's disease, offering substantial therapeutic implications.

Keywords: near-infrared laser diode, amyloid beta, neuroprotection, glial activation, necroptosis

P7-66

Therapeutic effects of Delta-9-tetrahyrocannabinol (Δ^9THC) as Alzheimer's disease pharmacotherapy: Behavioral and neuronal changes in hippocampus in induced rat model

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Alzheimer's disease (AD) is the most common form of dementia, an age-dependent neurodegenerative process characterised by gradual cognitive decline and neuronal deterioration. To date, the pharmacotherapies available for AD is still limited and only effective alleviating memory-declining symptoms. There is no existing approved-alternative that targets neurodegenerative processes or neuronal apoptosis at an early stage which may counteract the degradation process. Over the years, recreational and pharmacological properties of marijuana are known but only recently, cannabis has gained interest since endocannabinoid system (ECS) is highly expressed in the hippocampus and cortex, hence Δ^9THC often has been associated with memory and learning functions. In regards to this claim, this study has been designed to explore the potential therapeutic effect of Δ^9THC on cognition and histology in D-gal + $AlCl_3$ induced Wistar rats. In this study, male albino Wistar rats were exposed to 60 mg/kg D-gal intraperitoneally and 200 mg/kg $AlCl_3$ orally, once daily for 10 consecutives weeks. After 10 weeks, Δ^9THC at 1.5 mg/kg was administered for 28 days in the treatment phase. 1 mg/kg donepezil was used as positive control measure. Performance of the rats were evaluated through behavioral assessment; Morris Water Maze (MWM). Hematoxylin and eosin (H&E) staining was used to determine the apoptotic CA1 pyramidal cells and granule cells of dentate gyrus in the hippocampus. The results revealed that D-gal + $AlCl_3$ could significantly impair behaviour and cognitive function causing neurodegeneration by damaging CA1 pyramidal neurons in rats. Treatment of Δ^9THC conversely alleviated cognitive impairment by improving spatial learning and memory, reduced neuronal apoptosis and prevented morphological aberrations in CA1 region and dentate gyrus. Thus, the results suggest that Δ^9THC has therapeutic potentials act as a promising strategy that Δ^9THC may ameliorates AD-associated cognitive deficit.

Keywords: Alzheimer's disease, cannabis, THC, hippocampus, cognitive impairment

P7-67

Crif1 deficiency in dopamine neurons triggers early-onset parkinsonism by causing mitochondrial dysfunction

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Mitochondrial dysfunction has been implicated in Parkinson's Disease (PD) progression; however, the mitochondrial factors underlying the development of PD symptoms remain unclear. One candidate is CR6-interacting factor1 (CRIF1), which controls translation and membrane insertion of 13 mitochondrial proteins involved in oxidative phosphorylation. Here, we found that CRIF1 mRNA and protein expression were significantly reduced in postmortem brains of elderly PD patients compared to normal controls. To evaluate the effect of *Crif1* deficiency, we produced mice lacking the *Crif1* gene in dopaminergic neurons (DAT-CRIF1-KO mice). From 5 weeks of age, DAT-CRIF1-KO mice began to show decreased dopamine production with progressive neuronal degeneration in the nigral area. At ~10 weeks of age, they developed PD-like behavioral deficits, including gait abnormalities, rigidity, and resting tremor. L-DOPA, a medication used to treat PD, ameliorated these defects at an early stage, although it was ineffective in older animals. Taken together, the observation that CRIF1 expression is reduced in human PD brains and deletion of CRIF1 in dopaminergic neurons leads to early-onset PD with stepwise PD progression support the conclusion that CRIF1-mediated mitochondrial function is important for the survival of dopaminergic neurons.

Keywords: mitochondria, blood brain barrier, Notch1, ICH

P7-68

AST-001 improves social deficits by restoring dopamine neuron activity in VPA-induced ASD models

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Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by impaired social communication and social interaction, accompanied with restricted and repetitive behavior, interests or activities. The core symptoms of ASD are associated with deficits in mesocorticolimbic dopamine pathways that project from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) and medial prefrontal cortex (mPFC). There is no treatment for ASD core symptoms, and only a few medications are approved for the treatment of irritability associated with autism. Here we identify novel therapeutic agents for the treatment of ASD, called AST-001. In this study, we confirmed that oral administration of AST-001 was well delivered to brain regions by *in vitro* and *in vivo* tests. Furthermore, administrations of AST-001 im-

proved sociability and social novelty in the valproic acid (VPA)-induced mouse model. In VTA dopamine neurons of the VPA mouse model, abnormal spontaneous firing rates were normalized by administrations of AST-001 through rescuing HCN and SK channel activity. Overall, the findings suggest that AST-001 may be a potential therapeutic agent for ASD patients, and its mechanism of action may involve regulating dopamine neuron activity and rescuing social interaction.

Keywords: autism spectrum disorder, social interaction, dopamine neuron, SK channel, HCN channel

P7-69

WITHDRAWN

P7-70

The lateralization effect of dorsal insular cortex and rostral ventrolateral medulla on cardiovascular function in focal stroke

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The abnormal blood pressure was the common comorbidity in ischemic stroke patients. And the dorsal insular cortex (DIC) was the brain region that mostly involved in ischemic brain injury. The DIC was the one of central autonomic network (CAN), a groups of brain regions that responsible for autonomic nervous system modulation. However, the underlying mechanism in DIC damage led to aberrant blood pressure was still not clear. To verify the connection between DIC stroke and aberrant blood pressure, we established focal stroke model by unilateral endothelin-1 microinjection in DIC to induce local blood flow blockage in rats and recorded its blood pressure. The significant decreased blood pressure was recorded in the day 4 after right side DIC (DIC-R) focal stroke, but not in left side DIC (DIC-L) stroke. Besides, the DIC-R stroke model showed the defect of working and spatial memory. Furthermore, the power spectrum analysis showed the significant decreased power of very-low frequency (VLF) occurred in DIC-R stroke group. The VLF component represented neurogenic vasomotor control of blood vessel that originated from rostral ventrolateral medulla (RVLM), the region that response for sympathetic activity and cardiovascular function. It implied that RVLM activity was disturbed by DIC lesion. The directed neuron projection from DIC to RVLM was found in the FluoroGold labeling. Molecular analysis demonstrated the right side RVLM expression of *atg5* and *becn1*, molecules that regulated initiation stage of autophagy flux, were inhibited under ipsilateral DIC stroke. However, the left side RVLM molecular expression was affected by both ipsilateral and contralateral DIC lesion. These results showed that DIC-R had effect on both side RVLM and contributed to decrease blood pressure significantly. To verify the role of autophagy influx in blood pressure regulation after DIC stroke, we administrated rapamycin, the autophagy activator, in DIC-R stroke rats. The blood pressure was rescued and the power of VLF was enhanced. In conclusion, the DIC had function on cardiovascular regulation via modulating RVLM activity by neuron projection. While the laterization effect caused the different response of RVLM activity after unilateral DIC stroke, and this outcome was caused by the inhibition of autophagy function.

Keywords: ischemic stroke, dorsal insular cortex, rostral ventrolateral medulla, blood pressure, autophagy

P7-71

Investigation of curcumin treatment on neuropsychiatric symptoms and microstructural changes in a tuberous sclerosis complex mouse model

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Background: First, using *Tsc2*$^{+/-}$ knockout mice as a model, we aim to find the image-based phenotypes and their correlations with behavioral deficits in tuberous sclerosis complex (TSC). Second objective is to test whether the treatment of a natural mTOR inhibitor, curcumin, can improve the abnormal brain circuitry, as well as the neurocognitive deficiencies.
Methods: We used novel object recognition, open-field, and 3-chambered social behavior tests to assess the effectiveness of curcumin therapy. We next deployed region-based and tract-based diffusion-tensor imaging (DTI) to determine fractional anisotropy (FA), axial, radial, and mean diffusivities (AD, RD, and MD respectively) in several brain regions in *Tsc2*$^{+/-}$ mice. Finally, we used immunostaining assays and transmission electron microscopy to demonstrate the underlying molecular mechanisms of structural changes in the mutant mice.
Results: *Tsc2*$^{+/-}$ mice showed deficits in their behaviors. Also, they showed increased FA and decreased MD values in the region-based analysis, while decreased FA and AD values in tract-based analysis. Curcumin treatment reversed the abnormal behaviors and DTI measures. Using immunostaining of several proteins, we demonstrated that treatment with curcumin effectively lowered the expression of phospho-S6 and glial fibrillary acidic protein, and increased the expression of myelin basic protein. The complexity of myelinated axons was re-established, the expression level of the proteins was reversed, and the myelination was enhanced. These molecular changes corresponded with the alterations in DTI values.
Conclusion: Using DTI approach, we are able to characterize the image-based phenotypes in the *Tsc2*$^{+/-}$ mice and that these phenotypes correlated to the behavioral deficits. We also showed that targeting mTOR signaling by curcumin can change the myelination and hippocampal astrogliosis, thereby improving the behavioral deficiencies as well as the altered DTI values. This study, therefore, offers an alternative therapeutic approach for TSC with the identification of neuroimaging phenotypes as a guide for early diagnosis/prognosis to assess the neurocognitive improvement after treatments.

Keywords: tuberous sclerosis complex, diffusion tensor imaging, curcumin, neuropsychiatric disorders

P7-72

Global cerebral ischemia-induced depression associated with altered neuronal excitability in the infralimbic cortex layer 2/3 pyramidal neurons

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Cerebral ischemia can lead to a range of sequelae, including depression. The pathogenesis of depression involves neuronal change of the medial prefrontal cortex (mPFC). However, how cerebral ischemia-induced changes manifest across subregions and layers of the mPFC is not well understood. In this study, we induced cerebral ischemia in mice via transient bilateral common carotid artery occlusion (tBCCAO) and observed depressive-like behavior. Using whole-cell patch clamp recording, we identified changes in excitability of pyramidal neurons in the prelimbic cortex (PL) and infralimbic cortex (IL), the subregions of mPFC. Compared to sham control mice, tBCCAO mice showed significantly reduced neuronal excitability in IL layer 2/3 but not layer 5 pyramidal neurons, accompanied by an increase in rheobase current and a decrease in input resistance. In contrast, no changes were observed in excitability of PL layer 2/3 and layer 5 pyramidal neurons. Our results provide a new direction for studying the pathogenesis of depression following ischemic damage by showing that cerebral ischemia induces subregion- and layer-specific changes in the mPFC pyramidal neurons.

Keywords: cerebral ischemia, depression, medial prefrontal cortex, infralimbic cortex, excitability

P7-73

The NAD^+/NADH ratio regulates the invasion of Glioblastoma

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Glioblastoma(GBM) is the most aggressive type of cancer in the brain and has a very poor prognosis for survival. GBM has a different metabolic system, which strongly promotes motility, proliferation, and invasion. Among various metabolic pathways, NAD(nicotinamide adenine dinucleotide) is particularly important in generating ATP and is used as a resource for cancer cells. *Lb*NOX(*Lactobacillus brevis* NADH oxidase) is an enzyme that can directly manipulate NAD^+/NADH ratio and is one of the GEMMs(Genetically Encoded tools for Manipulation of Metabolisms), specifically converts NADH into NAD^+ in cells. In this study, we found that an increased NAD^+/NADH ratio reduced motility, proliferation, and invasion in GBM. Subsequent calcium imaging showed a decreased intracellular calcium response by *Lb*NOX or mito*Lb*NOX, and the endoplasmic reticulum calcium response was not affected. These findings suggest that the increased NAD^+/NADH ratio by *Lb*NOX or mito*Lb*NOX affected calcium signaling and consequently reduced motility, proliferation, and invasion.

Keywords: glioblastoma, GEMMs, NAD^+/NADH, invasion, calcium

P7-74

The modulation of mitochondrial function with increase of NAD/NADH ratio protects blood-brain barrier disruption in acute brain injury

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The blood-brain barrier (BBB) is composed of endothelial cells, astrocytes, and pericytes known as neurovascular unit. Endothelial cells have junctional proteins to maintain BBB integrity as a first line of barrier. Maintenance of BBB is essential to protect the brain from the infiltration of pathogens and prevent acute brain injury by oxidative stress. To date, the treatment of anticoagulants and thrombolysis has been used for stroke patients after the occurrence of the diseases. According to our previous reports, the maintenance of mitochondrial Oxphos in cerebral endothelial cells is critical for BBB integrity. But therapeutic agents for the maintenance of BBB and the prevention of BBB disruption still need to be developed. In this study, we found a supplement that can increase NAD/NADH ratio with enhancement of mitochondrial respiration before the induction of oxidative stress by oxygen-glucose deprivation (OGD) in the cerebral endothelial cells. Evans blue assay are performed after OGD, ischemic condition with the supplement pretreatment *in vitro* BBB model and Junctional proteins expression are investigated. Furthermore, we examined mitochondrial complex expression and mitohormetic response to investigate the effect of the supplement on mitochondrial functions. Pretreatment of the supplement in mouse intracerebral hemorrhage model alleviated the injury by increasing the junctional protein expression and mitochondrial modulation in cerebral endothelial cells. These results demonstrate that modulating mitochondria in cerebral endothelial cells can prevent BBB disruption.

Keywords: mitochondria, blood-brain barrier, endothelial cell, NAD/NADH ratio

P7-75

Promising effects of CBD-loaded nanolipid carriers in reduction of anxiety- and panic-like behaviors and hippocampal cell death in immobilization-stressed rats

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Anxiety disorders and related behavioral abnormalities, induced by stress exposure, are prevalent mental health conditions with signifi-

Poster

cant global societal impact. Cannabidiol (CBD), a non-psychoactive compound of Cannabis sativa, has emerged as a potential therapeutic agent for managing anxiety-related disorders. However, the limited bioavailability and poor solubility of CBD present challenges in achieving optimal therapeutic outcomes. Based on our recent findings found that nanotechnology-based delivery systems, i.e., nanolipid carriers, effectively enhance the bioavailability and targeted delivery of CBD. Therefore, this study aimed to investigate the effects of CBD-loaded nanolipid carriers on anxiety- and panic-like behaviors, as well as hippocampal cell death, in rats subjected to subchronic immobilization stress. Sexually male Wistar rats were subjected to daily 2 hours immobilization stress for 2 weeks to induce anxiety- and panic-like behaviors. The rats were divided into four groups: control (non-stressed, vehicle-treated), immobilization-stressed (vehicle-treated), immobilization-stressed + CBD (CBD-treated), and immobilization-stressed + CBD-loaded nanolipid carriers (CBD-nanolipid-treated) groups. CBD and CBD-loaded nanolipid carriers (10 mg/kg) were administered orally once daily for 2 weeks. Anxiety- and panic-like behaviors were assessed using the open field test, and elevated-T maze (ETM). Hippocampal cell death was evaluated by H and E staining and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. The result showed that administration of CBD-loaded nanolipid carriers significantly reduced anxiety- and panic-like behaviors compared to CBD-treated group, as demonstrated by lower total line crossed in open field test and escape latency in ETM, respectively. Moreover, CBD-nanolipid treatment significantly abolished hippocampal cell death, as demonstrated by the decreased number of pyknotic cells and TUNEL-positive cells. In conclusion, orally administered CBD-loaded nanolipid carriers show promise as a novel therapeutic application for alleviating anxiety- and panic-like behaviors and neuronal cell death in stressed male rats. These results suggest that the enhanced bioavailability and targeted delivery of CBD achieved through nanolipid carriers provide a potential avenue for optimizing CBD-based interventions in anxiety-related disorders. Further investigations are warranted to elucidate the underlying mechanisms and evaluate the long-term effects of this delivery system.

Keywords: cannabidiol, elevated-T maze, immobilization stress, nanolipids, panicolytic agent

Poster

P7-76

Alterations of mechanical bone properties in high emotionality stressed rats treated with atomoxetine

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Emotional stress has been recognized as a significant factor influencing bone health, yet the underlying mechanisms remain elusive. This study aimed to investigate the alterations in mechanical bone properties in highly emotionally stressed rats following treatment with Atomoxetine, a selective norepinephrine reuptake inhibitor. Eight-week-old male Wistar rats were subjected to immobilization in a modified plastic restrainer for 2 hours per day, 7 days per week, for 2 weeks, while a control group experienced no stress. Stressed rats were randomly divided into two groups: i) receiving daily oral administration of Atomoxetine (10 mg/kg) and ii) receiving a vehicle (5 mL/kg normal saline). Weekly sucrose preference was conducted to evaluate emotionality. Mechanical bone properties were assessed using a three-point bending biomechanical test. The results found that high emotionality stressed rats, as demonstrated by increased sucrose consumption. Furthermore, stressed rats exhibited significant impairments in femoral mechanical properties compared to the control group, characterized by decreased ultimate load, stiffness, and energy to fracture. Notably, stressed rats treated with Atomoxetine showed improvements in these mechanical parameters, with significantly higher stiffness and energy to fracture compared to the vehicle-treated group. Histological analysis by H&E staining further demonstrated that Atomoxetine treatment attenuated stress-induced alterations in bone morphology with increased numbers of osteoblasts. These findings suggest that Atomoxetine could ameliorate the negative effects of psychological restraint stress on bone integrity, potentially through its modulation of norepinephrine reuptake. Further studies could explore the precise molecular mechanisms underlying the observed effects and determine the long-term implications of Atomoxetine treatment on bone health in highly stressed individuals and mental illness patients.

Keywords: 3-points bending, bone strength, NRI, restraint stress, sucrose intake

P7-77

Vagus nerve mediates anxiety-like behavior in streptozotocin-induced type 1 diabetic mice

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Over 15% of type 1 diabetes (T1D) patients accompany anxiety-like behavior, even though T1D is due to the specific destruction of pancreatic beta cells. In addition, puncturing the fourth ventricle where the vagal afferent nerves innervate causes hyperglycemia-induced diabetes. Although crosstalk between the pancreatic beta cells and the brainstem in T1D has been reported, how they communicate through the vagus nerve and affect neurobehavioral change still needs more investigation. Firstly, we evaluated the anxiety-like behavior of Streptozotocin (STZ)-induced T1D mice by using the open field test (OFT), elevated plus maze (EPM) test, and light-dark transition test (LDT). STZ-induced diabetic mice displayed anxiety-like behaviors compared to vehicle mice which include, decreased time spent in the center in OFT, increased time in the closed arms in the EPM test, reduced frequency of the light-dark transitions in LDT, and decreased time in the light box in LDT. To examine the involvement of the vagus nerve in whether the anxiety-like behavior is elicited by the destruction of pancreatic beta cells, we performed ventral subdiaphragmatic vagotomy before intraperitoneal injection with STZ or vehicle. Subdiaphragmatic vagotomy of the ventral branch made no difference in the behavioral changes between the vehicle mice and STZ-induced diabetic mice. These results show that vagotomy can reverse anxiety-like behavior in STZ-induced diabetic mice. Taken together, the pancreas-brain axis is connected not only to the bloodstream but also to the nervous system which is through the vagal afferents.

Keywords: type 1 diabetes, anxiety, vagus nerve, mitochondria

P7-78

Enhancement of mitochondrial hormesis attenuates anxiety-like behavior

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Creatine transfers phosphate to ADP for supplying energy in brain. It has been reported that patients with post-traumatic stress disorder have low creatine levels. Moreover, the administration of creatine analogue to the mouse increased mitochondrial biogenesis in the brain cortex and alleviated anxiety. However, there is lack of investigation on the underline mechanism of enhancement mitochondrial function by the treatment of creatine analogue and how they affect neurobehavioral changes. To investigate anxiety-like behavior, we performed the behavioral test with open field test, elevated plus maze, light-dark box test after inhibition of creatine transporter by i.p. injection of creatine analogue. Interestingly, we found an increase in the time in open arms of elevated plus maze in creatine analogue treated mice comparing vehicle-treated groups. Since creatine transporter is primarily localized in brain endothelial cells, we analyzed the mitochondrial Oxphos system and mitochondrial hormesis-related proteins in isolation the microvessel from the mouse brain. As we expected, creatine analogue treatment increased mitochondrial respiration in isolated microvessel of creatine analogue treated group comparing vehicle-treated groups. Furthermore, creatine analogue administration altered the levels of mitochondrial UPR-associated protein expression which are the regulator of mitochondrial hormesis in the brain tissue. These results suggested that creatine analogue attenuates anxiety-like behavior through enhancement of mitochondrial hormesis and could be therapeutic agents for the mood disorder such as post-traumatic stress disorder.

Keywords: creatine analogue, creatine transporter, mitochondria, anxiolytic behavior

P7-79

Testing a novel NMDA receptor modulator for unmet medical needs in the treatment of schizophrenia: from preclinical models to clinical testing

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Excitatory neurotransmission mediated by N-methyl-D-aspartate receptor (NMDAR, a glutamate receptor) is primitive to and widely spread in mammalian central nervous system. It has been implicated in many fundamental functions and complex mental functions, including neuronal plasticity, learning, memory, and schizophrenia. Schizophrenia, a severe mental illness, affects 1% of population worldwide, including Taiwan. Common symptoms associated with schizophrenia include positive, negative, and cognitive symptoms. While existing agents treat positive symptoms of schizophrenia relatively well, negative and cognitive symptoms of schizophrenia have become an unmet medical need for antipsychotics development. In recent decades, increasing evidence from genetic, clinical, and pharmacological studies supports the NMDAR hypofunction hypothesis of schizophrenia. Blockade of the NMDAR can precipitate schizophrenic-like symptoms in both normal humans and animal models. Notably, several NMDA-enhancing agents, especially through glycine modulatory site (GMS) of NMDAR, resulted in a significant reduction of schizophrenia-related symptoms in patients. Particularly, D-amino acid oxidase (DAO) is now of great interest for the treatment of schizophrenia and several psychiatric disorders because its major substrate in the brain is D-serine, a co-agonist of the GMS on NMDAR. Accumulating data also provide clear evidence of increased cerebellar DAO (mRNA and enzyme activity) in schizophrenia. DAO inhibitor is one of the most attractive therapeutic targets for improving cognition and reducing negative symptoms in schizophrenia. Our inter-institutional research team has developed RS-D7 (a new chemical entity and a novel DAO inhibitor) for the treatment of negative and cognitive symptoms of schizophrenia for several years and obtained several awards. Given that NMDAR-mediated signaling pathway has been implicated in cognitive functions and that DAO inhibitors are potential therapeutic targets for enhancing activation of NMDARs, it is of great interest to investigate the effects of RS-D7 on the regulation of schizophrenia-related deficits and the underlying neural mechanisms in pharmacological (i.e., MK-801-induced) and genetic (i.e., serine racemase mutant) NMDAR hypofunction mouse models of schizophrenia. A series of cell-based assays and mouse studies was conducted and indicated efficacy, therapeutic effects, and underlying mechanism of RS-D7. Taking advantage of RS-D7 can be converted through a market drug RS-D7pro, our team further demonstrated RS-D7's significant therapeutic potentials in negative/cognition symptoms of schizophrenia and other neuropsychiatric disorders in our proof-of-concept clinical studies. Collectively, the therapeutic potentials of DAO inhibitors (especially RS-D7) on schizophrenia and other neuropsychiatric disorders are worth further investigation.

Keywords: schizophrenia, unmet medical need, NMDAR hypofunction, DAO inhibitor, novel drug

P7-80

Abnormalities in primary cilia formation through chronic social defeat stress may contribute to the onset of schizophrenia

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Chronic social defeat stress (SDS) is a leading cause of depression-like behaviors and mental disorders. SDS can alter the formation of primary cilia, which are non-motor sensory organelles that play a crucial role in cellular signaling and communication. Primary cilia dysfunctions can lead to a group of heterogeneous disorders called ciliopathies. These disorders include renal and liver cysts, retinal degeneration, and heart disease. Additionally, genes associated with ciliopathies are linked to neurological deficits such as autism spectrum disorder, schizophrenia, depression, intellectual disability, and epilepsy.
In this study, a mouse model of schizophrenia was established by chronic social defeat stress, and the relevance of primary ciliary regulation was studied. The relationship between primary cilia formation and ER stress or autophagy was verified. Antipsychotic administration was

found to regulate primary cilia formation in vitro, and inhibiting factors involved in primary cilia formation induced schizophrenic behavior in a mouse model. These findings suggest that aberrant expression of primary ciliary genes may be associated with the schizophrenic phenotype induced by chronic social stress, and regulating primary ciliary formation may lead to remission of schizophrenic symptoms.

Keywords: schizophrenia, social defeat stress, primary cilia, ER stress, autophagy

P7-81

Spontaneous hyperactivity does not limit electrically-evoked RGC spikes in degenerate retina

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Background: Hyperactive spontaneous spikes in retinal ganglion cells (RGCs) are a well-known characteristic of a degenerate retina. This spontaneous hyperactivity has been regarded as a reduced signal-to-noise ratio (SNR) which may limit the efficacy of implanted prosthesis in the degenerate retina. Our previous study investigated stage-dependent changes of RGC response to electrical stimulation in different ages of *rd10* mice. However, we have not probed in detail the possibility of reduced SNR to limit the electrically-evoked RGC spikes in the degenerate retina. This study compared the spike number of pre-electrical stimulus and post-stimulus to investigate if spontaneous hyperactivity limits the electrically-evoked spike number.
Methods: Spontaneous and electrically-evoked RGC spikes of *rd 10* mice rd10 (B6.CXB1-Pde6brd10/J) mice RGCs were recorded using 8×8 multi-electrode arrays (MEA) at postnatal week (PNW) 4.5, 6.5, 8, 10, 15, 20, 26, and 34. Retinal explants mounted on an MEA were stimulated by applying 1 Hz cathodic-phase first biphasic current pulses (Duration: 500 μs, Amplitude: 5, 10, 20, 30, 40, 50, 60 μA, 50 pulses per each amplitude, total 350 pulses). Evoked spike number was defined as the difference between spike number in post-stimulus 100 ms and pre-stimulus 100 ms. Pre-stimulus spike number was compared with the number of post-stimulus and evoked spikes, respectively.
Results: Spontaneous firing rate of RGC is higher in the early degeneration stage (PNW 4.6 & 6.5) and lower in the late stage (PNW 8~34) ($p<0.01$). The early stage showing higher pre-stimulus spike ($p<0.01$) also has higher post-stimulus spikes ($p<0.05$), resulting in no difference in evoked-spike number between early- and late-stage of RD ($p>0.05$).
Conclusion: We suggest that spontaneous hyperactivity in RD might not limit prosthetic efficiency to evoke RGC spikes. Therefore, retinal prostheses can be adapted regardless of the stage of retinal disease.

Keywords: retinal degeneration, retinal prosthesis, rd10 mouse, retinal ganglion cell, multi-electrode array

P7-82

The impact of stress on observational fear learning in mice

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Impairments in empathy have been observed in various neuropsychiatric disorders, including depression, psychopathy, and schizophrenia. Observational fear, describing as social transmission of fear from a demonstrator to an observer, is a form of affective empathy involving in social perception and cognitive processes. In observational fear, the inability to elicit a similar affective response in observers when witnessing distress in others is associated with lack of empathy. Early life stress (ELS) has been shown to increase the risk of developing mental illness, such as depression, by enhancing vulnerability to adult stress. Thus, we aim to investigate the impact of stress on observational fear. Using a two-hit stress models in mice, we divided them into four groups: control, ELS, adult chronic unpredictable stress (CUS) group, and double stress (DS; both ELS and CUS). Our findings demonstrated that the DS mice exhibited reduced freezing time, indicating impaired observational fear. However, the ELS and CUS groups did not exhibit decrease in freezing time. Mechanistically, we identified reduced neural activity in the basolateral amygdala (BLA) and the ventral hippocampus (vHP) CA1 in the DS mice. Further, neuronal tracing revealed that the vHP to BLA neural circuit was inhibited in the DS group. Activation of the vHP-BLA circuitry using designer receptor exclusively activated by designer drugs (DREADD) improved fear response in the DS mice during the observational fear paradigm. Overall, our results suggest that early life stress along with chronic unpredictable stress triggers observational fear impairment through inhibition of the vHP -BLA pathway.

Keywords: stress, observational fear, basolateral amygdala, ventral hippocampus

P7-83

Social network index, perceived stress scale and geriatric depression scale among elderly persons in magway township, Myanmar

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Background: The population of elderly persons is about 10% of the total population in Myanmar, and it is expected to grow rapidly. The risk of stress and depression is likely to be high among elderly persons. A social network is important for elderly persons to attain mental well-being. Therefore, the present study aims to study the Social Network Index (SNI), Perceived Stress Scale (PSS) and Geriatric Depression Scale (GDS) among elderly persons in Magway Township.
Methodology: A community-based cross-sectional comparative study was conducted on six hundred elderly individuals aged ≥60 years. The participants were randomly selected from wards and villages in Magway Township. Face-to-face interviews were conducted at their homes by well-trained interviewers. The social activity, stress level and depression status of the elderly persons were assessed using the Social Network Index, Perceived Stress Scale and Geriatric Depression Scale, respectively. Physical fitness and mental status were screened using the Activities of Daily Living and Mini-Cog™.
Results: Out of the 600 subjects,18.4% of them reported a limited social network, 30.5% experienced average to high perceived stress, and 11.2% had depression. The 35% of the subjects were from urban areas, while 65% were from rural areas. Social network was better in rural areas; however, the proportion of subjects experiencing perceived stress and depression was also high in rural areas. There was a significant negative correlation between SNI and GDS ($r= -0.2028$, $n = 600$, $p<0.001$), as well as a significant positive correlation between PSS and GDS ($r= 0.5842$, $n = 600$, $p<0.0001$) within the study population. However, there was no significant correlation between SNI and PSS ($r= -0.0725$, $n = 600$,

p>0.05).
Conclusion: Generally, the proportion of depression was 11.2% in the elderly population, and it was not significantly different between rural and urban areas. Although there was a better social network, the perceived stress level and proportion of depression were higher in rural areas. This could be attributed to the lower education level and lower socio-economic status in rural areas. The overall effect of the social network on depression was significant; however, the perceived stress level had a stronger effect on depression.

Keywords: social network index, perceived stress scale, geriatric depression scale, elderly persons

P7-84

Optogenetic gamma stimulation of primary somatosensory cortex modulate neuronal activity in the spinal dorsal horn and induce allodynia-like behavior in mice

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Primary somatosensory cortex (S1) is one of the main regions in the cerebral cortex to receive and process pain information conveyed from the periphery. It has been shown that cortical oscillations especially in the gamma frequency band (30-100 Hz) in the S1 are related to pain in both humans and rodents. In a recent study, it has been shown that 40 Hz optogenetic entrainment of parvalbumin-positive (PV) neurons localized in S1 (S1-PV-gamma-activation) enhanced nociceptive sensitivity and induced aversive behavior in mice (Tan et al., Nat Comm 2019). It was indicated that these effects are mediated through the descending serotonergic pain modulation system. However, although the activity of spinal dorsal horn (SDH) neurons is also known to be modulated by these descending inputs, the effect of S1-PV-gamma-activation on the SDH neuron activity remains unclear. In this study, we investigated the temporal changes of SDH neuron activity and the nociceptive behavior during and after the S1-PV-gamma-activation in transgenic mice expressing channelrhodopsin (ChR2) in PV neurons (PV-ChR2 mice). Extracellular single-unit recording *in vivo* was performed to examine the effect of S1-PV-gamma-activation (40 Hz, 5-s stimulation, 20-s interval, 5 cycles) on SDH neurons activity in urethan-anesthetized PV-ChR2 mice. There was no significant change in spontaneous firing in all the neurons examined (n = 7). However, the firing rate significantly increased (approximately 2-fold) in response to punctate mechanical force (0.4/4.0 g) to the planter surface of the hindpaw contralateral to the activated cortical side with von Fry filament. The effect was transient and the response returned to baseline after 30 min. To examine the nociceptive behavior, we performed von Frey test in freely moving mice up to 30 minutes after S1-PV-gamma-activation in the left S1. Non-noxious filament (0.07 g) was applied to the planter surface of the hindpaw on both sides. During activation, withdraw response in right hindpaw significantly increased but not in the left one in PV-ChR2 mice. In the majority (5 out of 9) of the mice examined, the increased response persisted for more than 5 min after the activation. Our results indicate that S1-PV-gamma-activation induces allodynia and hyperalgesia by increasing the firing rate of SDH neurons.

Keywords: primary somatosensory cortex, gamma oscillation, pain, spinal dorsal horn, neuronal activity

P7-85

Central angiotensin II modulates different pain pathway through its two receptors

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Background: Although accumulating evidences have indicated importance of central angiotensin (Ang) II in the nociceptive progressing, underlying mechanisms of central Ang II in the modulation of nociceptive transmission in the orofacial area remains uncertain. We examined the underlying mechanisms of mechanical allodynia and thermal hyperalgesia induced by intracisternal injection of Ang II using male Sprague-Dawley rats.
Methods: Anesthetized male Sprague-Dawley rats were fixed in a stereotaxic instrument. A very small hole was made in the atlanto-occipital membrane and dura and the end of the PE tube was inserted. The cannula tip of the PE was placed at the obex level of the medulla. To evaluate mechanical allodynia, the withdrawal behavior responses were observed including escape from air-puff stimulation. To evaluate thermal hyperalgesia, the head withdrawal latency time was measured after radiant heat application. The co-localizations of Ang II receptors were performed by double immunostaining.
Results: Intracisternal injection of Ang II produced significant mechanical allodynia and thermal hyperalgesia compared to the vehicle-treated rats. Intracisternal injection of Ang II receptor antagonists was performed 2 h after Ang II injection. Losartan, an Ang II type 1 receptor (AT1R) antagonist, attenuated mechanical allodynia but not thermal hyperalgesia. Conversely, PD123319, an Ang II type 1 receptor (AT2R) antagonist, blocked only thermal hyperalgesia. Immunofluorescence analyses revealed co-localization of AT1R with the astrocyte marker GFAP and co-localization of AT2R with CGRP-positive neurons in the trigeminal ganglion. Intracisternal pretreatment with minocycline, a microglia inhibitor, did not affect Ang II-induced mechanical allodynia, while LAA, an astrocyte inhibitor, significantly inhibited Ang II-induced mechanical allodynia. Moreover, subcutaneous pretreatment with botulinum toxin type (BTX)-A significantly attenuated Ang II-induced thermal hyperalgesia, but not Ang II-induced mechanical allodynia.
Conclusion: Central Ang II-induced mechanical allodynia is conveyed by AT1R in astrocytes, and Ang II-induced thermal hyperalgesia is conveyed by AT2R in the central terminals of CGRP-positive primary afferent neurons. These results suggest that central Ang II-induced nociception is differentially regulated by AT1R and AT2R. Thus, distinct therapeutic targets must be regulated to overcome pain symptom caused by multiple underlying mechanisms

Keywords: angiotensin II, angiotensin II receptor, neuropathic pain, botulinum toxin type A

Poster

P7-86

Peripheral Gpr35 activation modulates dermatitis and pruritus

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Pruritic dermatitis is a disease that poses a significant unmet need for effective treatment options and is characterized not only by epidermal manifestations but also by peripheral neuronal complications. To address this challenge, we propose a novel pharmacological approach targeting both peripheral dorsal root ganglion (DRG) sensory neurons and skin keratinocytes. Specifically, we focus on GPR35, an orphan G-protein-coupled receptor expressed in DRG neurons, which has been predicted to downregulate neuronal excitability upon activation. Recent research has shown that pamoic acid, a salt-forming agent for drugs, can selectively activate GPR35. In this study, we evaluated the effects of pamoic acid on dermatitis pathology. Our findings demonstrate that local application of pamoic acid mitigates acute non-histaminergic itch and consistently inhibits DRG neuronal responses. Furthermore, pamoic acid incubation reduces keratinocyte fragmentation under dermatitis simulation. Notably, repeated application of pamoic acid moderately but significantly reverses chronic pruritus in models of 1-chloro-2,4-dinitrobenzene (DNCB)-induced dermatitis and of psoriasis and improves dermatitis scores in these models. Collectively, our results suggest that peripheral GPR35 activation with pamoic acid holds promise for ameliorating pruritus and associated dermatologic conditions.

Keywords: GPR35, pamoic aicd, pruritus, dermatitis

P7-87

Multiple downstream signals of Gpr83 activation differentially control nociceptor excitabilities

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The modulation of pain by G protein-coupled receptors (GPRs) expressed in peripheral nociceptors is an important mechanism controlling pain transmission. Enhancing our comprehension of the roles of these receptors and anticipating their potential outcomes can promote the development of new analgesic strategies. In this study, we have identified a previously unrecognized GPR regarding pain modulation, Gpr83, with relatively high expression in the peripheral nervous system. We demonstrate that silencing Gpr83 expression in murine dorsal root ganglia resulted in the downregulation of both neuronal and behavioral nociception. Furthermore, hind paw inflammation and chemotherapy-induced peripheral neuropathy were alleviated by Gpr83 knockdown. In vitro experiments revealed that the endogenous Gpr83 ligand PEN had variable effects on nociceptor responses depending on the duration of exposure. Localized administration of PEN in vivo mitigated pain, possibly by downregulating GPR activity involving Gq/11. These results suggest that Gpr83 is involved in the modulation of peripheral pain sensitivity and may hold promise as a target for pain management.

Keywords: GPR83, pain, PEN

P7-88

Pharmacologically inhibiting Il6st/gp130 results in improvement of dermatological inflammation and pruritus

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Dermatitis currently lacks effective pharmacological treatments. Cytokines play a key role in maintaining and exacerbating inflammation in dermatitis. The interleukin 6 signal transducer (Il6st), also known as glycoprotein 130 (Gp130), is important for receiving and amplifying cytokine signals. Based on this, we hypothesized that inhibiting Il6st could improve dermatitis. We tested two small molecule Il6st inhibitors, which bind to Il6st in different ways, in an animal model of 1-chloro-2,4-dinitrobenzene-induced dermatitis. Both treatments led to moderate but significant improvement in skin conditions, with one of the drugs appearing to normalize cytokine expression related to the dermatitis index. Pruritic behaviors were also reduced, potentially contributing to the improvement. In a psoriatic skin and itch model using imiquimod, the two treatments had a relatively moderate effect. Overall, inhibiting Il6st appears to alleviate pathological irritation, and our experiment suggests that Il6st is involved in the pathological mechanisms of dermatitis. Therefore, we propose Il6st as a novel target for improving dermatitis and that drugs with suitable efficacy and safety for its modulation could be translated into clinical use.

Keywords: Il6st, gp130, pruritus

P7-89

Neuromodulatory effect of zerumbone on alpha-$_{2A}$ adrenoceptor, NMDA N2B receptor and TRPV1 channel in *in silico* and *in vitro* model of neuropathic pain

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Neuropathic pain, caused by the lesion of the somatosensory nervous system, involve neuroinflammation and neuronal hyperexcitability. The occurrence of neuropathic pain in the general population is approximated to range from 3% to 17%. Although the condition is common, due to its association with diabetic condition or chemotherapy, the conventional therapies for neuropathic pain often have adverse effects due to the limited understanding of the underlying mechanisms. However, recent research suggests that zerumbone, a compound derived from *Zingiber zerumbet*, can alleviate neuropathic pain symptoms. This study examined the antineuropathic pain-effect of zerumbone on the descending modulation, through the regulation of α-$_{2A}$ adrenoceptors, N-methyl-D-aspartate (NMDA) subtype N2B receptors, and transient receptor potential vanilloid subtype 1 (TRPV1) channels.

This was achieved through *in silico* and *in vitro* models, via molecular docking and Western blot analysis respectively. Molecular docking analysis showed that zerumbone had a higher -CDOCKER binding and interaction energy with the target proteins compared to native ligands. Additionally, zerumbone exhibited agonistic interaction against α-$_{2A}$ adrenoceptor, and antagonistic interaction against NMDA N2B and TRPV1 channel. In *in vitro* model, LPS induction in differentiated SH-SY5Y cells mimicked the pathophysiology of neuropathic pain, characterized by the expression of pro-inflammatory mediators and changes in receptors and ion channels associated with neuronal hyperexcitability. Western blot analysis revealed that zerumbone significantly increased the expression of α-$_{2A}$ adrenoceptors while down-regulating the expression of NMDA N2B receptors and TRPV1 channels, as compared to the control groups. Data from each experiments were analysed by using One-way Analysis of Variance (ANOVA) followed by *post hoc* Tukey test, $p<0.05$. In conclusion, this study demonstrated the neuromodulatory effects of zerumbone on α-$_{2A}$ adrenoceptors, NMDA N2B receptors, and TRPV1 channels through molecular docking analysis and Western blot analysis on LPS-induced SH-SY5Y cells, providing evidence for its potential as a treatment for neuropathic pain.

Keywords: neuropathic pain, zerumbone, α-2A adrenoceptor, NMDA N2B receptors, TRPV1 channels

P7-90

Multiplexed representation of itch and pain and their interaction in the primary somatosensory cortex

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Itch and pain are distinct sensations that share anatomically similar pathways: from the periphery to the brain. Over the last decades, several itch-specific neural pathways and molecular markers have been identified at the peripheral and spinal cord levels. Although the perception of sensation is ultimately generated at the brain level, how the brain separately processes the signals is unclear. The primary somatosensory cortex (S1) plays a crucial role in the perception of somatosensory information, including touch, itch, and pain. In this study, we investigated how S1 neurons represent itch and pain differently. First, we established a spontaneous itch and pain mouse model. Spontaneous itch or pain was induced by intradermal treatment with 5-HT or capsaicin on the lateral neck and confirmed by a selective increase in scratching or wiping-like behavior, respectively. Next, *in vivo* two-photon calcium imaging was performed in awake mice after four different treatments, including 5-HT, capsaicin, and each vehicle. By comparing the calcium activity acquired during different sessions, we distinguished the cells responsive to itch or pain sensations. Of the total responsive cells, 11% were both responsive, and their activity in the pain session was slightly higher than that in the itch session. Itch- and pain-preferred cells accounted for 28.4% and 60.6%, respectively, and the preferred cells showed the lowest activity in their counter sessions. Therefore, our results suggest that S1 uses a multiplexed coding strategy to encode itch and pain, and S1 neurons represent the interaction between itch and pain. In our ongoing studies, we are comparing how itch and pain are represented differently between facial and hind paw skin. Additionally, we are investigating the changes in S1 during the development of chronic itch.

Keywords: itch, pain, somatosensory cortex, in vivo two photon imaging

P7-91

Amyloid beta 1-42 modulates heat pain sensitivity in aged mice through TRPV1 inhibition via the LRP1-SHP2 pathway

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With aging, there is an observed impairment in the ability to perceive heat, leading to risks such as an increased propensity for burns, compromised thermoregulatory function, and reduced ability to recognize signs of physical disorders, including infections and inflammation. This study aims to elucidate the mechanisms underpinning these alterations in heat sensitivity, providing a basis for strategies to manage associated risks.

We investigated the role of amyloid beta (Aβ), a substance that accumulates with aging in peripheral tissues, in modulating heat sensitivity. Our findings indicate that aged mice show attenuated heat pain responses compared to younger ones at temperatures (45°C and 50°C) known to activate transient receptor potential vanilloid 1 (TRPV1). Subsequent in vivo and in vitro experiments demonstrated that Aβ1-42 inhibits TRPV1 activation. Interestingly, we identified the role of the Low-density lipoprotein receptor-related protein-1 (LRP1) and Src homology region 2-containing protein tyrosine phosphatase 2 (SHP2) pathway in mediating this inhibitory effect of Aβ1-42 on TRPV1.

Notably, Aβ1-42 administration attenuated heat hyperalgesia in the spared nerve injury (SNI) model, via the LRP1-SHP2-TRPV1 pathway, implying therapeutic potential. Collectively, our findings underscore the complex interplay between Aβ1-42, TRPV1, LRP1, SHP2, and thermal pain modulation, which may have significant implications for managing neurogenic pain in chronic pain patients with related conditions.

Keywords: aging, pain, TRPV1, amyloid beta, LRP1

P7-92

The role of IL-33 in the trigeminal ganglion in orofacial neuropathic pain

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Neuropathic pain is an intractable pain caused by nerve or tissue damage. Numerous studies have shown that various pro-inflammatory cytokines potentiate neuronal activity in the trigeminal ganglion (TG), leading to neuropathic pain in the orofacial region. We previously identified that interleukin (IL)-33 is a novel cytokine involved in orofacial neuropathic pain at the trigeminal spinal subnucleus caudalis level. However, the role of IL-33 in neuropathic pain remains not fully understood. Here, we show IL-33 in the TG is required for orofacial neuropathic pain in C57BL/6J mice. The head withdrawal threshold (HWT) in response to von Frey filament stimulation to the whisker pad skin decreased after infraorbital nerve injury (IONI). On the other hand, HWT was unchanged after the sham operation. Five days after IONI, a significant increase in IL-33 was observed in the TG of IONI mice compared to that of sham mice. IL-33 was predominantly expressed in fibroblasts but not in neurons,

macrophages, and satellite glial cells in the TG after IONI. And its receptor was expressed in TG neurons. Mechanical allodynia in the whisker pad skin was suppressed by neutralization of IL-33 in the TG of IONI mice. Conversely, intra-TG administration of IL-33 elicited mechanical allodynia in naïve mice. IL-33-induced mechanical allodynia was suppressed by pharmacological inhibition or gene silencing of TRPA1 in the TG. These results suggest that fibroblast-derived IL-33 sensitizes TRPA1 in TG neurons, leading to the development of mechanical allodynia in the orofacial region.

Keywords: Il-33, neuropathic pain, trigeminal ganglion, TRPA1, mechanical allodynia

P7-93

Analgesic mechanisms of linalool odor on oral ulcerative mucositis-induced pain in rats

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Background: Oral ulcerative mucositis (OUM) induces severe pain. It's important to relieve pain to improve the QOL in OUM patients. Linalool odor exposure has recently been reported to suppress inflammatory pain in the hind paw. However, the analgesic effect of linalool odor on orofacial pain is unclear. Here, to elucidate whether linalool odor ameliorates OUM-induced pain, we investigated the analgesic effect of linalool odor using behavioral and immunohistochemical analyses in the OUM model rats.
Methods: OUM was developed by treatment with acetic acid in the labial fornix region of the inferior incisors. Linalool at 1% (0.53 ppm) was exposed to rats for 5 min at 30 min before behavioral observation. To determine whether the effect of linalool odor exert via olfaction and descending inhibitory system, 2% lidocaine was applied around the olfactory bulb 3 min before linalool exposure, and an orexin receptor OX-1 antagonist was administered into the locus coeruleus (LC) 30 min before linalool exposure, respectively. The number of c-Fos-positive neurons was assessed in the trigeminal spinal subnucleus caudalis (Vc) following linalool odor exposure in the OUM model rats. Motor function was evaluated using Rota-rod.
Results: Compared with naive rats, the spontaneous and the capsaicin-induced rubbing times were prolonged, and the mechanical head-withdrawal threshold in the oral mucosa was decreased in the OUM model rats. These nocifensive behaviors were suppressed by linalool odor without affecting motor function. The number of c-Fos positive neurons in Vc of OUM model rats was decreased by linalool exposure. Both lidocaine application to the olfactory bulb and administration of OX-1 antagonist into the LC inhibited the decrease in spontaneous rubbing time following linalool odor exposure in the OUM model rats.
Conclusion: These results suggest that linalool odor exerts an analgesic effect on OUM-induced pain via activation of orexin neurons in the locus coeruleus.

Keywords: pain, oral ulcerative mucositis, linalool, olfactory bulb, locus coeruleus

P7-94

Modulation of brain-derived neurotrophic factor expression: investigating the effect of physical exercise on reserpine-induced pain and depression-like responses in mice

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Pain accompanied by depressive symptoms is a common cause for seeking medical assistance, with approximately 35% of chronic pain patients experiencing comorbid depression. Brain-derived neurotrophic factor (BDNF) is a well-known neurotrophin that is expressed throughout the peripheral and central nervous systems, playing a role in neuronal growth and neuroplasticity. This study aimed to examine the effect of exercise and the involvement of BDNF in reserpine-induced pain and depression-like responses in mice. To induce pain and depression-like behavior, mice were subcutaneously administered reserpine (RSP, 1 mg/kg) once daily for three days. The exercise was performed using a rota-rod tester for seven consecutive days following RSP injection. Pain behavior responses were evaluated using von Frey filaments on the hind paws, while depression-like behaviors were assessed through forced swimming and open field tests. Immunofluorescence staining was conducted on dorsal root ganglions (DRG) and the spinal cord to examine peripheral and central BDNF expression changes. Repeated RSP injections led to mechanical hypersensitivity, increased immobility time in the forced swimming test, and reduced movement in the open field test. Immunofluorescence results revealed a significant increase in BDNF expression in the DRG and spinal dorsal area following RSP administration. Exercise effectively mitigated abnormal sensory responses and depression-like behaviors induced by RSP. Furthermore, exercise suppressed the heightened expression of BDNF in the DRG and spinal dorsal area. These findings suggest that repetitive exercise could serve as an effective and non-invasive treatment option for individuals experiencing both pain and depression by modulating BDNF expression.

Keywords: analgesia, exercise, brain-derived neurotrophic factor, fibromyalgia, reserpine

P7-95

Local lymphadenopathy-mediated neuro-immune mechanisms in vincristine-induced peripheral neuropathy

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Chemotherapy-induced peripheral neuropathy (CIPN), which frequently occurs in anti-cancer drug treated patients, manifests chronic pain with distinct gloves and stockings patterns, implying chemotherapeutic damages on long peripheral axons. Vincristine, one of the most neurotoxic anti-cancer agents, is also reported to develop the typical pattern of terminal neuropathy along with infiltration of activated immune cells into long peripheral axons. However, it still remains to be elucidated why and how long axons are more susceptible to vincristine-induced peripheral neuropathy. In current study, I hypothesized immune activation in the lymph nodes adjacent to the long-axon is responsible for

pain development and immune cell infiltration into the sciatic nerve after vincristine administration. To verify the hypothesis, I confirmed a development of vincristine-induced pain in mouse model through application of von Frey filament into the hind paw after vincristine administrations. 4 days after the first administration of vincristine, lymph nodes were extracted for immunohistochemical and flow cytometrical examinations. In vincristine-treated mice, I found the lymphadenopathy in axillary and sciatic lymph nodes which are located adjacent to long axon innervating arms and legs, while surgical removal of the sciatic lymph node not only reduced pain development but also prevented infiltration of macrophage and CD4+ T cells into the sciatic nerve after vincristine administration. Populations of activated T cells and NK cells were increased both in the sciatic lymph nodes and peripheral blood, and in vivo depletion of CD4+ T cells or NK cells significantly reduced development of the vincristine-induced mechanical allodynia and also reversed upregulation of cytokine CXCL13 in sciatic nerve after vincristine treatment. Immunohistochemical analysis also revealed increased expression of CXCL13 in vincristine-treated sciatic nerve, while the depletions of NK and CD4 T cell reversed CXCL13 expression. Additionally, in vivo administration of anti-CXCL13 antibody mixed Matrigel around the sciatic nerve prevented the development of mechanical pain after vincristine treatment in dose dependent manner. In conclusion, the findings suggest the local immune activation in the long-axon adjacent lymph node is responsible for vincristine-induced pain development via activation of NK and T cells resulting pain generating molecule, CXCL13, in the sciatic nerve.

Keywords: neuropathic pain, chemotherapy, lymph node, CXCL13

P7-96

Alleviation of painful diabetic peripheral neuropathy in mice through activation of TREK-1 channel: inhibiting sensory neuron and Schwann cell dysfunction

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Painful peripheral neuropathy is a common complication in diabetes, but the mechanisms and treatment options for painful diabetic peripheral neuropathy (pDPN) are not fully understood. Hyperglycemia and metabolic stressors affect sensory neurons (dorsal root ganglion neurons, DRGs) and Schwann cells (SCs), potentially causing changes in ion channel expression and peripheral sensitization. Although TREK channels have been studied for their potential in pain relief, their specific role in pDPN and their impact on SCs remain unclear. This study aims to investigate the involvement of TREK channels in a mouse model of pDPN, with a specific focus on the function of DRGs and SCs. A pDPN model was created using a high-fat diet (HFD, 60 kcal %) and streptozotocin (60 mg/kg). Both TREK-1 and TREK-2 channels were detected in the DRGs, but their expression levels were significantly diminished in the pDPN group compared to the control group. Notably, TREK-1 channels were primarily expressed in SCs, with higher levels observed in SCs compared to DRGs. Furthermore, the expression of TREK-1 was reduced in SCs obtained from the pDPN group. Sciatic nerves obtained from the pDPN model exhibited higher levels of cell death of SCs and macrophage infiltration compared to the control group. In isolated SCs, exposure to a high concentration of glucose resulted in decreased TREK-1 activity and expression level. This decrease in TREK-1 expression was associated with cell death, which was attenuated in cells overexpressing TREK-1. Knockdown of TREK-1 in SCs led to reduced cell proliferation and migration compared to SCs transfected with scrambled siRNA. Treatment with TREK-1 inhibitors reduced the proliferation and migration of SCs. Moreover, TREK-1 knockdown and inhibition in SCs exposed to glucose triggered the secretion of inflammatory mediators and reactive oxygen species, contributing to nerve damage and pain in pDPN. These findings suggest that TREK-1 may play a role in promoting peripheral nerve regeneration by preserving the function of DRGs and SCs in pDPN.

Keywords: DRG, Schwann cell, TREK-1, TREK-2, diabetic periperal neuropathy

P7-97

Tactile hypersensitivity relies on Piezo1 in nociceptors during inflammation

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The process of somatic touch sensation is initiated when pressure directly stimulates mechanically activated (MA) channels in the nerve endings of dorsal root ganglion (DRG) neurons. Piezo1, a fast-inactivating MA channel, has surfaced to be involved in touch and pruriception. However, the specific mechanisms involved in mechanical pain are still unknown. Here, we employed fluorescence *in situ* hybridization, calcium imaging, whole-cell patch-clamp recordings, and behavioral tests in mice to identify the critical role of Piezo1 in mechanical pain transmission. Our findings demonstrate that Piezo1 is expressed in a subset of DRG neurons associated with nociceptors that are positive for Trpv1 rather than Mrgprd. To better understand the physiological functions of Piezo1 in DRG neurons, we classified them based on the type of MA currents; intermediately adapting and intermediately slowly adapting responses were significantly reduced by Piezo1 shRNA. We also observed a reduction in inflammation-induced hypersensitivity through ganglionic injection of Piezo1 shRNA viruses in mice. Taken together, our results suggest that *Piezo1* mediates mechanical pain by acting as a nociceptive MA channel in inflammation.

Keywords: dorsal root ganglion neurons, Piezo1, tactile hypersensitivity, inflammatory pain

P7-98

Development of sympathetically-maintained pain following peripheral nerve injury depends on the type of nerve injury

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Sympathetic nervous system is potentially involved in pain hypersensitivity after peripheral nerve injuries, which has been demonstrated

in several animal models for neuropathic pain. In this study, we aimed to identify the type of nerve injury that leads to sympathetically-maintained pain (SMP). We compared two different neuropathic pain models, L5 spinal nerve transection (L5 SpNT) and partial crush injury (PCI), which represent neurotmesis and axonotmesis models, respectively. Involvement of the sympathetic nervous system was confirmed by examining sympathetic nerve sprouting by immunofluorescence staining using tyrosine hydroxylase (TH) and measuring norepinephrine (NE) release using ELISA in the dorsal root ganglia (DRG). In addition, we examined whether tactile hypersensitivity is attenuated by chemical sympathectomy with systemic administration of 6-hydroxydopamine (200 mg/kg) before the surgery. We found that TH-positive nerve fibers and the level of NE were only increased in lumber DRGs after L5 SpNT but not after PCI. Besides, chemical sympathectomy significantly reversed tactile hypersensitivity in L5 SpNT, but not in PCI. Furthermore, when we examined whether the distance of nerve injury from DRG is critically involved in the sympathetic sprouting to L4 DRG in both crush and transection injuries of L4 spinal nerve and sciatic nerve, we observed that TH-positive fibers were increased in L4 DRG after both sciatic nerve transection (ScNT) and L4 spinal nerve transection (L4 SpNT), but not after PCI and L4 spinal nerve crush (L4 SpNC). Taken together, our results demonstrate limited involvement of sympathetic nervous system in crush-induced neuropathy condition rather than in transection injury condition, regardless of the location of nerve injury. This finding suggests that the type of nerve injury is a critical factor for the development of SMP.

Keywords: neuropathic pain, sympathetically-maintained pain, sympathetic nerve sprouting, partial crush injury, spinal nerve transection

P7-99

The orexin a pathway antinociceptive effect on formalin-induced acute pain in intermittent fasting model

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Various factors such as hunger, noise, and the exposure to cold can cause stress and the stress response may induce analgesia by elevating some stress-related hormones. We designed this study to determine whether learning stress can elicit antinociception without a stress response. As a stress factor, we chose fasting by grouping two different conditions such as acute fasting (AF) as a non-predictable stress group and intermittent fasting (IF) as a programmed stress group. In addition, we analyzed the orexin A (OXA) neuronal activity of the hunger center, lateral hypothalamus (LH), and the blood level of corticosterone (CORT) as a major stress hormone. In the present study, AF group mice were fasted for 6, 12, or 24 hours before formalin test while IF group mice were fasted for 12 hours/12 hours or 24 hours/24 hours fasting/eating sequences. For the acute pain model, we injected formalin solution (1%, 20ul) into the plantar surface of the right hind paw, recorded the pain behavior for 40-minutes, and measured the licking time (sec). Except for 6 hours AF, all mouse groups showed antinociception in the formalin test. We demonstrated the analgesic effects mediated by increased orexin A in the spinal cord of the stress-programmed IF12hr group through staining and Western blot analysis of OXA, fos-B, c-Fos, orexin 1 receptor (OR1), and other markers. In conclusion, 12 hours of IF may produce a significant antinociception on formalin-induced pain without CORT elevation and this result suggests IF may have a high potential as a pain treatment.

Keywords: orexin a, stress induced analgesia, intermittent fasting, programmed stress, pain

P7-100

Piezo1 down-streaming TRP activation in odontoblasts

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Direct mechanical stimulation to the odontoblasts, that are dentin forming and mechano-sensory receptor cells, activates not only the transient receptor potential (TRP) channel subfamilies (TRPV1, TRPV2, TRPV4 and TRPA1), but also mechanosensitive ion channel, Piezo1 channels (Piezo1), leading intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) increases. Recently, in pancreatic acinar cells or endothelial cells, Piezo1 is capable to regulate mechanosensitive TRPV4 activation via intracellular signaling pathway. In odontoblasts, however, detailed mechanism of such interaction among Piezo1 and TRP channel subfamilies expressed in odontoblasts has remained to be clarified. The present study aimed to investigate the interaction machinery between Piezo1 and TRP channel subfamilies in acutely isolated rat odontoblasts by fura-2 fluorescence measurements. Application of Yoda1 (1.5 min in duration), a pharmacological Piezo1 activator, to odontoblasts elicited biphasic increases in $[Ca^{2+}]_i$. When we applied Yoda1 and Dooku1 (antagonist for the Yoda1-evoked responses) simultaneously, both the initial and sustained phase of Yoda1-induced biphasic responses were significantly inhibited. Long-lasting application of Yoda1 (10 min in duration) induced persistent increases in $[Ca^{2+}]_i$, which were inhibited by A784168, TRPV1 antagonist. We suggested that Piezo1 functionally interacts with TRPV1 in odontoblasts.

Keywords: Piezo, TRP, odontoblast, oral physiology, dentistry

P7-101

Reduced oxidative stress alleviates chemotherapy-induced peripheral neuropathy via improving mitochondrial biogenesis

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Chemotherapy-induced peripheral neuropathy (CIPN) is a frequent dose-limiting side effect and a major cause of persistent pain in cancer patients with chemotherapy. Although many treatments have been developed currently, there is still no effective strategy for preventing CIPN. Previous studies of vinpocetine, a phosphodiesterase 1 inhibitor, can reduce oxidative stress and inflammatory cytokines. However, the role of vinpocetine in CIPN remained unclear. In this study, we investigated the analgesic effect of vinpocetine treatment and related mechanisms in CIPN.
A mice model of CIPN was established by paclitaxel (2 mg/kg, i.p.) for 4 alternate days, and the treatment of vinpocetine was applied for 7 days after the end of paclitaxel injection. von Fray test and Hargreaves

test were used to evaluate mechanical allodynia and thermal hyperalgesia, respectively. Western blot was used to examine mitochondrial biogenesis-related proteins in the spinal cord and dorsal root ganglia (DRG). To confirm mitochondrial function, ROS levels were detected and analyzed.
In our study, repetitive administration of vinpocetine could attenuate CIPN symptoms. The expression of mitochondrial biogenesis-related proteins was restored, and ROS production was decreased after vinpocetine treatment.
We demonstrated the analgesic properties of vinpocetine in CIPN. It suggests that mitochondrial biogenesis with vinpocetine could be a novel therapeutic target of CIPN treatment.

Keywords: CIPN, oxidative stress, mitochondrial dysfunction, mitochondrial biogenesis, vinpocetine

P7-102

The morphometric alteration of disc and bone induced chronic low back pain in rats

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Degenerative changes in the intervertebral disc are recognized as one of the critical causes of low back pain (LBP). Bulging or herniation of degenerative disc compress the adjacent spinal nerves or spinal cord, transmitting mechanical compression-related pain signals. However, there has been less explanation for whether extensive degeneration in disc and bone, without severe irritation of herniated disc, can affect pain processing. We aim to investigate whether two types of intradiscal injuries alter spine structure and induce behavioral alteration and hyperexcitability of peripheral neurons in rats. We performed puncture/nucleus pulposus aspiration (PUNCT), involving mechanical injury with nucleus pulposus (NP) removal, and monosodium iodoacetate (MIA)-injected discs, involving chemical injury with nucleus pulposus (NP) presence, which were compared to sham-surgery and saline-injected disc group. The structure of the disc/bone was analyzed using μCT and immunohistochemistry. NGF and BDNF expression within disc/subchondral bone and animal behavior were assessed for nociceptive processing. PUNCT showed significantly larger intervertebral space (IVS) volume by local bone erosion with hypertrophic trabecular bone. MIA showed significantly lowered disc height and smaller IVS volume with weakened trabecular bone. Degeneration groups expressed significantly increased NGF and BDNF in disc/subchondral bone. Degeneration groups showed behavioral changes including significant increases in forelimbs dependency on weight bearing and the sensitivity of the hindlimb. Our current study suggests that morphometric changes in disc/bone contribute to neuronal and behavioral hypersensitivity associated with pain.

Keywords: low back pain (LBP), intervertebral disc (IVD), micro-computed tomography (μCT), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF)

P7-103

Molecular docking and molecular dynamic simulation of cardamonin on the voltage-gated sodium channel 1.7

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Neuropathic pain is a debilitating condition affecting millions of individuals worldwide, often with limited treatment options and significant side effects. Addressing this need, the present study investigates the potential of cardamonin, a plant-based chalcone, as a novel therapeutic agent for neuropathic pain management. While previous research has demonstrated the analgesic properties of cardamonin, the precise molecular mechanism underlying its effects remains unclear. This study aims to shed light on the interaction between cardamonin and the voltage-gated sodium channel subtype 1.7 (NaV1.7) through in-silico modelling techniques, specifically molecular docking, and molecular dynamics simulations. Molecular docking experiments were performed utilizing Autodock Vina, with cardamonin (Pubchem CID: 641785) as the ligand and NaV1.7 (PDB ID: 5EKO) as the receptor. The results revealed multiple potential binding conformations of cardamonin to NaV1.7, encompassing the pore binding site and voltage-sensing domain four (VSD-4). Among these conformations, the most favourable binding occurred at the VSD-4 binding site, exhibiting a Gibbs free binding energy of -7.8 kJ. To evaluate the stability of the cardamonin-NaV1.7 complex at VSD-4, a subsequent molecular dynamics simulation was conducted using GROMACS over a period of 200 nanoseconds. The simulation results indicated the sustained stability of the cardamonin-NaV1.7 complex throughout the entire duration. Further analysis of the simulation data revealed notable interactions, including Pi-anion interactions with ASP 1586, Pi-Pi T-shaped interactions with TRP 1538 and TYR 1537, and Pi-Alkyl interactions with PHE 1598 and ARG 1605. Collectively, these findings suggest a potential binding mechanism between cardamonin and NaV1.7, providing insight into the compound's analgesic properties. In conclusion, the present study identifies the VSD-4 binding site as the most favourable interaction site for cardamonin with NaV1.7, demonstrating stability over a 200ns molecular dynamics simulation. These findings pave the way for further investigations into cardamonin's effects on other binding sites and for extended simulation durations to comprehensively elucidate its mechanisms. Moreover, these results serve as a foundation for evaluating the impact of cardamonin on NaV1.7 in animal models, potentially facilitating the development of novel neuropathic pain treatments.

Keywords: cardamonin, voltage-gated sodium channel 1.7, neuropathic pain, molecular docking, molecular dynamic simulation

P7-104

Na^+ influx and T-type Ca^{2+} channel activation by monosodium urate on the terminal of substantia gelatinosa neurons in juvenile mice

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Monosodium urate (MSU), known as the etiology of acute inflammatory gout, is deposited around the joints and in soft tissues, causing inflammation and excruciating pain. Recently, various biological effects of MSU on the central nervous system have been studied. However, there have been no reports of the activity of MSU in orofacial nociceptive pain modulation. The substantia gelatinosa (SG) of the trigeminal subnucleus caudalis (Vc) is known to be a central site that integrates and modulates afferent fibers that transmit the orofacial nociceptive information. In this study, the whole-cell patch-clamp was applied to examine the role of MSU and its action mechanism on the SG neurons of the Vc in juvenile mice. Under the high chloride, low chloride, or cesium chloride pipette solution, MSU was applied alone or with antagonist (TTX, CNQX, AP-5, picrotoxin, strychnine, mibefradil, or nimodipine) in ACSF or choline chloride bath solution. *A* relative comparison was used to estimate the response of MSU alone or with reagents based on changes of the inward currents. The effect of MSU on the SG neurons was observed the inward current in the high chloride pipette solution in ACSF solution. Additionally, MSU-induced inward current was reduced in the low chloride pipette solution in choline chloride bath solution, but the inward current was still induced. However, the inward current was significantly decreased by mibefradil, a T-type calcium channel blocker in cesium chloride pipette solution. These results indicate that MSU affects sodium influx in SG neurons to increase intracellular sodium concentration and also affects T-type calcium channels, but additional research is needed. Taken together, the pain mechanisms of MSU contribute toward at least a part of orofacial nociceptive modulation and might be a promising target to develop therapeutic agents in orofacial pain treatment.

Keywords: substantia gelatinosa neurons, orofacial nociception, monosodium urate, sodium influx, calcium channel

P7-105

Gut microbiota and related metabolites are involved in the development of vincristine-induced peripheral neuropathy

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Chemotherapy-induced peripheral neuropathy (CIPN) is a debilitating adverse side effect of cancer treatment. Current understanding of the mechanisms underpinning CIPN is still limited and unclear. The gut is connected to the CNS by immunological mediators, lymphocytes, neurotransmitters, microbes and microbial metabolites. There are many reports that microbiome and related metabolites exert significant effects on neurological diseases. However, the potential contribution of microbiota and related metabolites in CIPN has not been well reported. In the present study, we elucidated the role of gut microbiota and metabolites in CIPN. The chemotherapeutic agent, vincristine, produces a robust painful neuropathy that results in mechanical allodynia and the loss of intraepidermal nerve fibers (IENFs). Vincristine-induced mechanical allodynia and loss of IENFs were significantly reduced in mice pretreated with antibiotics. We investigated metabolic changes in serum of mice treated with vincristine and antibiotics (vinc+antibio). Interestingly, high level of tryptophan metabolite was found in serum with vinc+antibio group. Moreover, pretreatment with tryptophan itself suppressed vincristine-induced mechanical allodynia and loss of IENFs. In conclusion, these results suggest that gut microbiota and related metabolites may be involved in the development of vincristine-induced peripheral neuropathy, and tryptophan could novel strategy for CINP management.

Keywords: chemotherapy, microbiota, neurodegeneration, neuropathy, pain

P7-106

Inhibitory action of magnolol on substantia gelatinosa neurons of the trigeminal subnucleus caudalis in mice

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The substantia gelatinosa (SG) of the trigeminal subnucleus caudalis (Vc) regulates orofacial nociceptive information via primary afferent fibers. Although magnolol, a major active constituent extracted from the bark of *Magnolia officinalis*, has possessed a variety of biological effects on humans including antinociception, the action mechanism of magnolol on SG neurons of the Vc has not been fully clarified. In this study, we investigated the direct membrane effects of magnolol on SG neurons of the Vc in immature ICR mice using the whole-cell patch-clamp technique. The application of magnolol exerted non-desensitizing and repeatable inward currents which were remained in the presence of tetrodotoxin (a voltage gated Na^+ channel blocker) and in CNQX (a non-NMDA glutamate receptor antagonist) and AP5 (an NMDA receptor antagonist), suggesting that magnolol may act directly on the postsynaptic SG neurons. Moreover, magnolol-mediated inward currents were significantly reduced in the presence of strychnine (a glycine receptor antagonist) or picrotoxin (a $GABA_A$ receptor antagonist). Altogether, our results indicate that magnolol may be a target molecule for orofacial pain regulation through the activation of glycine and/or $GABA_A$ receptors on the SG region.

Keywords: substantia gelatinosa neurons, magnolol, patch clamp, glycine receptors, GABAA receptors

P7-107

Alpha-lipoic acid does not affect GABA, glycine or glutamate receptor activities of substantia gelatinosa neurons of the trigeminal subnucleus caudalis in mice

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Substantia gelatinosa (SG; lamina II) neurons of trigeminal subnucleus caudalis form synapses with primary afferent nerves, and are key sites for receiving and modulating pain signals from the orofacial region and transmitting them to upper brain center. Alpha-lipoic acid (ALA), which is naturally synthesized in the body, is known to protect cells from oxidative stress as a powerful antioxidant. In addition, recent reports have demonstrated that ALA is effective in regulating orofacial pain such as burning mouth syndrome. However, little has been studied on what mechanisms of action ALA controls orofacial pain at the central nervous system (CNS) level. In our previous study, ALA acted directly on SG neurons, but the action was not mediated by $GABA_A$ receptor, glycine receptor or glutamate receptor. Therefore, in this study, we further investigated how ALA affects inhibitory or excitatory neurotransmitters reactivity of SG neurons using a whole-cell patch-clamp technique to understand the mechanism of action of ALA at the CNS level. Under the condition of high chloride pipette solution, pretreatment with ALA (100 uM) did not have any effect on the inward currents induced by GABA or glycine, which are representative inhibitory neurotransmitters in the CNS. Moreover, inward currents induced by glutamate, which is an excitatory neurotransmitter, was not significantly changed by ALA (100 uM). These results indicate that ALA does not affect the regulation of GABA, glycine or glutamate receptor activities in SG neurons, suggesting that pain modulation by ALA at the CNS level may be caused through other mechanisms rather than GABA, glycine or glutamate receptors.

Keywords: GABA, glycine, glutamate, orofacial pain, patch-clamp

P7-108

Intracellular cAMP-induced Ca^{2+} Influx via Activation of Protein Kinase A in Odontoblasts

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Odontoblasts are sensory receptor cells that detect a variety of stimuli applied to dentin surface. The stimuli activate mechanosensitive ion channels in odontoblasts, resulting in Ca^{2+} influx from extracellular medium. The intracellular Ca^{2+} signaling in odontoblasts plays critical roles in reactionary dentin formation and/or generating dentinal pain. In the previous study, we revealed that increase in intracellular cAMP level via activation of cannabinoid 1 receptors induced Ca^{2+} influx through transient receptor potential vanilloid subfamily member 1 in odontoblasts. These results suggest that intracellular cAMP level mediates Ca^{2+} signaling in odontoblasts. In this study, we investigated the crosstalk between intracellular Ca^{2+} and cAMP signaling in human odontoblasts.
We measured intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) and cAMP level using fura 2-AM and mNeonGreen-based cAMP sensor in human odontoblast (HOB) cells.
In the presence of extracellular Ca^{2+}, application of forskolin, an adenylyl cyclase activator, or isoproterenol, a beta-2 adrenergic receptor agonist, increased intracellular cAMP level and $[Ca^{2+}]_i$ in HOB cells. When we removed extracellular Ca^{2+}, forskolin- or isoproterenol-elicited $[Ca^{2+}]_i$ increase could not be observed. Application of a protein kinase A inhibitor suppressed forskolin elicited $[Ca^{2+}]_i$ increase in HOB cells. The $[Ca^{2+}]_i$ increase was also inhibited by application of a non-selective Ca^{2+} channel antagonists, $ZnSO_4$ or $GdCl_3$, or non-specific TRP channel antagonist, 2APB, while not inhibited by blockers of L- or T-type voltage dependent Ca^{2+} channels. In addition, forskolin-induced increase in intracellular cAMP level was not inhibited by application of $ZnSO_4$, or blockers of L- or T-type voltage dependent Ca^{2+} channels.
These results suggested that activation of adenylyl cyclase evoked increase in intracellular cAMP level and Ca^{2+} influx from extracellular medium via activation of protein kinase A in odontoblasts. The activation of the beta-2 adrenergic receptors increased intracellular cAMP level by stimulating adenylyl cyclase in odontoblasts. The Ca^{2+} influx induced by activation of adenylyl cyclase was mediated by Zn^{2+}-, Gd^{3+}-, and 2APB-sensitive Ca^{2+} channels, with the exception of L- and T-type voltage dependent Ca^{2+} channels.

Keywords: odontoblasts, cyclic AMP, calcium

P7-109

The inflammatory cytokine, interleukin-1-beta drives central immune cell infiltration, changes in sympathetic nerve activity and blood pressure

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Background: The area postrema (AP) is a brain region that lacks a blood brain barrier allowing blood-borne factors to access the brain. Since the AP is connected to numerous brain regions that play a key role in blood pressure regulation, we hypothesised that blood-borne inflammatory factors such as interleukin-1 beta (IL-1b) might play a role in the development of hypertension. In support of this hypothesis, plasma Interleukin-1 beta is increased in hypertensive patients (Dalekos et al., 1997, J Lab Clin Med,129: 300-8). Furthermore, the receptors for IL-1b are abundantly expressed in the AP.
Aims: Test whether IL-1b can act on the AP to cause an increase in blood pressure and renal sympathetic nerve activity (RSNA) in rats.
Methods: Blood pressure and renal sympathetic nerve activity (RSNA) were recorded and analyzed after microinjection of different doses of IL-1b into the AP (10ng and 25ng) of anaesthetised rats. In order to show that IL-1b, specifically causes an increase in blood pressure, specific IL-1 receptor blocking antibodies (IL-1Ra) were injected into the AP prior to a 25ng IL-1b injection.
Results: Experiments showed that microinjection of 25ng IL-1b caused a significant ($p<0.01$; Mann-Whitney test) increase in both systolic blood pressure (19.6 ± 3.6 mmHg) and heart rate (55 ±10 bpm) compared with control injections of saline (SBP: 5.47 ± 1.58; HR 12 ± 6). Pre-treatment with IL-1Ra attenuated the response to IL-1b, and was comparable with saline controls. IL-1b also caused an increase in RSNA (137% ± 43%). Immunohistochemistry also revealed that IL-1b injection results in Fos activation in the AP.
Conclusion: IL-1b injection appears to activate neurons in the area postrema which leads to an increase in blood pressure. By providing new insight into the actions of IL-1b in the brain, we show that IL-1b might be a potential target for future treatments for hypertension.

Keywords: blood pressure, cytokines, sympathetic nerve activity, area postrema, immune cells

Poster

P7-110

Hypothalamic TRPM2-expressing POMC neurons-mediated control of BAT thermogenesis

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The hypothalamus is a key brain region that controls feeding and energy expenditure. It plays a major role in regulating BAT thermogenesis by generating heat via adrenergic receptor-mediated mitochondrial processes. Thus, understanding how hypothalamic neurons, which are associated with energy balance, regulate BAT thermogenesis via neuronal connection is important. Here we demonstrate that the role of TRPM2-expressing hypothalamic POMC neurons influences BAT thermogenesis. TRPM2 agonist ADPR increases neuronal activity of POMC neurons, consequently generating heat in BAT, which is neuronally connected to POMC neurons. However, the thermogenic effect of ADPR is blocked by TRPM2 antagonist clotrimazole. Among the BAT thermogenic markers, PRDM16 is increased by ADPR. In parallel, ADPR increased IRF-4 levels in BAT. Taken together, our findings indicate that TRPM2-mediated activation of hypothalamic POMC neurons is critical for the elevation of BAT temperature, determining the strength of energy expenditure.

Keywords: hypothalamus, brown adipose tissue, thermogenesis, POMC, TRPM2

P7-111

The impacts of the cholinergic system on acute and chronic arthritis

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The autonomic nervous system (ANS) consists of the sympathetic and parasympathetic nervous systems, which are balanced by mutual antagonism. Some studies reported that the cholinergic system within the ANS can alleviate pain in various diseases and has anti-inflammatory effects through the cholinergic anti-inflammatory pathway (CAIP). However, it is not clear whether the cholinergic system similarly regulates pain and inflammation in knee arthritis.
The purpose of this study was to investigate the knee joint pain and inflammation changes brought about by the cholinergic system on acute and chronic arthritis.
Sprague Dawley rats (200-250g; Korea) were used. Acute and chronic arthritis models were induced by injecting 1% λ-carrageenan (50μL) and CFA (100μL), respectively, into the left knee joint cavity of rat. It were divided into experimental (treated with atropine) and control groups (treated with saline), and pain-related behavior was observed by performing the Dynamic Weight Bearing test for 3-4 repetitions. Synovial membranes were collected from each group and the expression levels of pro-inflammatory cytokines such as COX-2 and IL-1β were measured using a western blot technique. Additionally, Hematoxyline & Eosin (H&E) staining were conducted to confirm histological changes of acute and chronic knee arthritis model rats.
Compared to saline-injection group, after 10 μL atropine application, pain worsened in both acute and chronic knee arthritis rat models. In addition, it was also shown that 10 μL atropine increased the expression level of cyclooxygenase-2 (COX-2) and interleukin-1β (IL-1β), and there was a significant increased in the number of inflammatory cells in synovial membrane. Therefore, the blockade of the cholinergic system exacerbated pain and inflammation on acute and chronic arthritis.
The present study implicate that the cholinergic system of the autonomic nervous system is involved in the pain and inflammatory processes of the knee joint and regulates them.

Keywords: autonomic nervous system (ANS), cholinergic system, knee joint pain, inflammation

P7-112

Comparison of the activated sweat gland density according to PNS and CNS responses

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The aim of this study was to quantitatively evaluate the difference in sudomotor function between the central nervous system (CNS) response and the peripheral nervous system (PNS) reflex by measuring the activated sweat gland density (ASGD) in their early twenties. For the quantification of ASGD due to the CSN response, a heat exposure test (40°C half-bath, 30 minutes) stimulating the CNS was used. CNS response was performed by immerging the lower legs in hot water (40° C) for 30 minutes. However, quantitative sudomotor axon reflex test (QSART), a method for evaluating autonomic nervous system activity, was used to quantify ASGD due to reflexes in the PNS. In QSART, the sweat glands are activated directly or indirectly by subcutaneous application of a neurotransmitter such as 10% acetylcholine via iontophoresis (2 mA * 5 min). This mechanism is called the sudomotor axon reflex. In both experiments, activated sweat glands appearing as blue-black pigmented spots on iodine-impregnated paper were counted three times in an area of 0.5 cmX0.5 cm under a microscope, and the average sweat gland density (count/cm2) was calculated. And the sweat gland density was measured and recorded on the eight regions of the skin (chest, upper arm, upper back, lower back, abdomen, thigh, forearm, and calf).

Keywords: quantitative sudomotor axon reflex test (QSART), central nervous system (CNS), activated sweat gland density (ASGD), peripheral nervous system (PNS), acetylcholine (ACh)

P7-113

The effect of caffeine contained in coffee to enhance the activation of sweating motor function

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The purpose of this study was to investigate whether the consumption of high-caffeinated coffee stimulates the sympathetic nerve response

to acetylcholine. We used the quantitative sudomotor axon reflex test (QSART), a standard component of clinical autonomic function testing to assess the integrity of the cholinergic segment of the sympathetic nervous system (SNS). The experiment was conducted in a climatic chamber at 25.0±0.5°C, 60.0±3.0% relative humidity, and 1 m/s wind speed. And QSART was performed before the start and after a 40-minute break after drinking coffee and water. In QSART, neurotransmitters such as 10% acetylcholine are used to quantify sweat secreted from sweat glands, and they are activated directly or indirectly by iontophoresis(2mA * 5min). After coffee consumption, it was confirmed that SNS activation through stimulation of acetylcholine affects the increase of AXR(1), AXR(2) and DIR sweat responses. It could be implied that coffee consumption may have an effect that causes caffeine to enhance activation of sweating and motor function.

Keywords: caffeine, axon-reflex, sympathetic nervous system(SNS), acetylcholine(ACh), quantitative sudomotor axon reflex test(QSART)

P7-114

Kinematics analysis by deep learning in the recovered forelimb by rehabilitation after hemorrhagic stroke in rats

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A switch from the cortico-spinal pathway to the cortico-rubral pathway, leading to functional recovery, was observed by rehabilitative training of forced limb use (FLU) after intracerebral hemorrhagic stroke (ICH) in rats (J. Neuroscience 36,455-67:2016). Although success rate of the single pellet reaching test was improved by FLU, it is still unknown whether quality changes of skilled reaching are shown by the rehabilitative training after ICH. To analyze detail kinematics of the forelimb function, markerless pose estimation using DeepLabCut based on deep neural network was applied to ICH model rats that had selective blockade of cerebello-rubral tract with DREADD system. AAV-DJ-EF1a-DIO-hM4D(Gi)-mCherry was injected into the cerebellar lateral nucleus and FuG-E-MSCV-Cre was injected into the parvocellular red nucleus, followed by electrophysiological confirmation: clozapine-N-oxide (CNO) administration decreased the responding current in both FLU group and normal rat, however CNO did not affect the success rate of skilled reaching in normal. Detail kinematics analysis revealed that mean distance between fingertip 1 to 4 became smaller with negative correlation to success rate in non-FLU-ICH group. In addition to significant increase in the success rate, positive relationship between the mean distance and success rate was observed in FLU-ICH group. Interestingly, the blockade of the cerebello-rubral tract with CNO significantly reduced the success rate, keeping the positive correlation. Further analysis of the finger shape revealed that the quality of finger movements significantly differs between non-FLU-ICH group and FLU-ICH group. Data suggest that the cerebello-rubral tract is related to the recovery of skilled forelimb function by FLU and that quality changes of skilled reaching are also induced by rehabilitative training by FLU after stroke.

Keywords: reaching, kinematics, cerebellum, DepLabCut, red nucleus

P7-115

The effects of chemotherapy on human taste

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The prevalence of chemotherapy-induced taste disorders occurs in approximately 60% of patients. Symptoms of taste disorder, such as decreased taste sensitivity and spontaneous unpleasant bitter or metallic tastes, have been widely reported. However, a great deal of variation among individuals and most of these reports are based on subjective symptoms of patients. In addition, no objective indices, such as taste tests for each basic taste, have been examined in previous studies. In this study, we conducted objective taste tests before and after chemotherapy.

Twenty-two breast cancer patients (mean age, 54.4; range, 32-79 years) were tested using the Saxon test as well as an electrogustometer, a four-basic taste discrimination test using the drop method of the whole-mouth gustatory test procedure. All data were collected for each subject before chemotherapy and after one and three courses of chemotherapy. The taste solutions for the four basic tastes were: sucrose for sweet, sodium chloride for salty, tartaric acid for sour, and quinine hydrochloride for bitter.

The thresholds measured by the electrogustometer showed no change before or after chemotherapy in the anterior part of the tongue, but the taste sensitivity in the posterior part of the tongue increased. Analysis of each taste quality demonstrated that sweet and salty tastes were less affected by chemotherapy than sour and bitter. Sour taste was affected after one course of chemotherapy and had a particularly high percentage of increased taste sensitivity, but the effect decreased after three courses. Bitter taste was more affected after three courses than after one course; specifically, the percentage of increased taste sensitivity to bitter was higher after three courses than that after one course. According to the Saxon test, salivary was not significantly different before or after chemotherapy.

In conclusion, the effects of chemotherapy on the sense of taste differed depending on the site on the tongue and the taste quality of each of the four basic tastes. These data suggest that, while it is generally believed that chemotherapy decrease taste sensitivity, they may also increase it in some instances.

Keywords: taste, chemotherapy, taste disorder

P7-116

An information of worker's 5-httlpr (slc6a4 gene) variants data; a challenge of behavior- physiological science application at workplace in the future

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Background: Genotype variants of the serotonergic system are involved in influencing work performance among workers. This variant has a role in driving behavior based on its function to control serotonin levels. Serotonin levels at extracellular fluid or synapses affect the emotional, cognitive, and behavior of workers.

Methods: This is an original work a systematic literature review serves as preparation of a study proposal with the purpose to explore the information of transporter serotonin gene variant-related work as a

consideration in formulating labor management policies at work in Bali-Indonesia.
Results: The serotonin transporter gene plays a role in encoding transporter serotonin (5-HTT) protein which functions to increase serotonin reuptake. The short allele (s/s allele) of the serotonin transporter gene (with lower mRNA transcriptional activity in the promoter gene than the long allele) increases the risk for a variety of psychological disorders, including depression, anxiety, and alcoholism. Previous studies found that a worker's sensitivity to stressors at work and the risk of work-related mental illness are correlated with differences in genotype/polymorphism variants of the serotonin transporter promoter gene. Availability of 5-HTTLPR (SLC6A4 Gene) variants data among workers is important for the organization/company, as a consideration for worker placement and in preparation of an occupational mental health management program to increase productivity and to build a better company image.
Conclusion: The availability of genotype data of the variant 5-HTTLPR (SLC6A4 Gene) among workers at a company in Bali-Indonesia is a challenge in the future related to the application of behavior physiology in the workplace as a basis for conducting human resource (HR) management program and company's policy.

Keywords: serotonergic system, mental health, workplace, 5-HTTLPR, stress sensitivity

Poster

P7-117

The effect of prenatal exposure to high cortisol on oxidative signaling of neurons in prefrontal cortex of rats

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Previously, we reported that prenatal exposure to high cortisol induced attention deficit-hyperactivity disorder (ADHD)-like behaviors with cognitive deficits after weaning. The cellular mechanism underlying this phenomenon seems to be attributed to the downregulation of dopaminergic and PKA-mediated signaling cascades during neurodevelopment, triggering the pathogenesis of neuropsychiatric disorders such as ADHD. Mitochondrial dysfunction has been reported to be one of critical causes, which are observed in neurodevelopmental disorders, because neurons have more abundant mitochondria than other cell types.
In the present study, we investigated the cellular functions to regulate oxidative signaling in cortical neurons isolated from prefrontal cortex of rat pups (Corti.Pups) which were born from rat mothers that were repetitively injected with corticosterone (s.c 20mg/kg/day, 21 days) during pregnancy. In this experiment, the differences in intracellular ROS, mitochondrial ROS production, mitochondrial membrane potential, and intracellular calcium level in dissociated PFC neurons were respectively compared between the normal group (Nor.Pups) and Corti.Pups using a fluorescent dye with a microplate reader. The oxygen consumption rate (OCR), a key mitochondrial parameter, was measured directly with the Seahorse XFe24 analyzer in intact neuron and isolated brain mitochondria. The results indicate that prenatal exposure to high cortisol strongly alters cellular functions to regulate oxidative signaling and neuronal survival in PFC neurons. This dysregulation of oxidation may be a critical factor of pathogenesis to be correlated with neurodevelopmental psychiatric disorders such as ADHD. (Grant No. NRF-2022R1I1A3063177).

Keywords: mitochondrial ROS, oxygen consumption rate, mitochondria

P7-118

The association between internet addiction and dry eyes during the COVID-19 pandemic among Indonesia medical students

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Background: In this digital era, the internet has become a part of human life. During the Covid-19 pandemic, the number of internet users is also increasing. Various pleasures and education activities offered by using the internet put internet users at risk of experiencing internet addiction. Excessive internet use causes internet addiction and adversely affects our bodies, including dry eyes. Dry eyes can cause discomfort that interferes with daily activities. Through this study, it hopes that there will be increased attention to the risks of internet addiction and its effects, especially dry eyes. This study aims to determine the prevalence of internet addiction, dry eyes, and the association between internet addiction and dry eyes among medical students.
Methods: This study uses a cross-sectional approach. Sampling was taken from July to August 2021 on 160 respondents aged 18-20 of the School of Medicine and Health Science Atma Jaya Catholic University of Indonesia. Quantitative data retrieval using a questionnaire through the G-form. The questionnaires used included a demographic data questionnaire, the Internet Addiction Test (IAT) to measure internet addiction, and the Ocular Surface Disease Index (OSDI) to measure the level of dry eye. Data were analyzed using the chi-square test with a value sign significance p<0,05.
Results: The prevalence of internet addiction is mild addiction 43,1%, moderate at 23,1%, and severe at 0,6%. Most dry eyes are mild 21,3%, moderate 10,6%, and severe 9,4%. There is a significant association between internet addiction and dry eyes (p=0,003).
Conclusion: The prevalence of internet addiction and dry eyes among medical students is relatively high, and there is an association between internet addiction and dry eyes. Based on the results of this study, prevention can be taken in the health sector to reduce the prevalence of internet addiction and dry eyes among medical students.

Keywords: internet addiction, dry eyes, medical students, Covid-19 pandemic, Indonesia

P7-119

Regulator of lipid metabolism PPARα/NHR-49 mediates *C.elegans* pathogen avoidance and precise control of neuronal activity

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Precise control of neuronal activity is crucial for the proper function of the nervous system. How lipid homeostasis contributes to neuronal activity and how much of it is regulated cell autonomously is unclear. In this study, while investigating the role of the ubiquitously expressed PPAR functional ortholog NHR-49 in the neurons of *C. elegans*, we found that mutants display deficient pathogen avoidance response. This behavior required NHR-49 in the neurons, and more specifically, in a set of oxygen-sensing body cavity neurons, URX, AQR, PQR. Calcium imaging in URX neurons using O_2 as the stimulus revealed that *nhr-49*

mutants displayed altered calcium kinetics, which correlated with lawn avoidance behavior. Supplementation with oleic acid improved lawn avoidance as well as calcium kinetics, suggesting that defective calcium handling in *nhr-49* neurons is due to lipid dysfunction, and points to possible NHR-49 targets that may be involved in maintaining calcium homeostasis. We provide evidence of calcium dysregulation in an additional neuron, the HSN motor neuron, suggesting that NHR-49 is likely required in many neurons to ensure calcium homeostasis. These findings highlight the role of cell-autonomous lipid regulation in neuronal physiology and organismal behavior.

Keywords: C.elegans, PPAR, behavior, neuroscience, NHR

P7-120

Transient prenatal ruxolitinib treatment promotes neurogenesis and suppresses astrogliogenesis during Ts1Cje mouse brain development

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Ruxolitinib is a Janus kinase (JAK) inhibitor approved by the FDA to treat myeloproliferative neoplasms. This drug suppressed JAK/STAT signalling pathway by inhibiting JAK1 and JAK2, which is important for modulating the gliogenesis process. In Down syndrome (DS), the JAK-STAT signalling was overstimulated, resulting in elevated *Gfap* and *S100β* expression, markers for astrocytes in the brain. The imbalance between neuronal and astrocyte brain cells was one of the causes of intellectual disabilities and impaired cognitive functions in DS. In the study, Ts1Cje, a DS mouse model, was used to assess the effect of ruxolitinib (0–30 mg/kg/day between E7.5 and E20.5) on the developing mouse brain. During the treatment, the pregnant mice did not show any adverse effects of the drug. The whole brain of P1.5 pups was harvested, followed by RNA extraction. Then, RT-qPCR was performed to determine several markers for neurogenesis and astrogliogenesis. The expression of *Gfap* (~263-fold) and *S100β* (~4-fold) was reduced in the ruxolitinib group compared to the control. In contrast, the treated group demonstrated an increase in the expression of neuronal markers, *vGLuT1* (~3-fold), *vGluT2* (~126-fold), and *GAT1* (~28-fold), compared to the control group. Moreover, in the treated group, the expression of neural progenitor/stem cell markers, *Nanog* (~3-fold), *Oct4* (~4-fold), *Sox1* (~2-fold), and *NeuroD1* (~2-fold) increased, while *Nestin* (~8-fold) expression decreased. These findings demonstrated the transplacental treatment of ruxolitinib during the developing brain modulates and regulates key markers involved in neuronal differentiation. These findings also suggest the potential use of ruxolitinib to revert pathological conditions caused by an imbalance of neuronal-astrocyte ratio in early brain development, such as Down syndromes.

Keywords: ruxolitinib, JAK-STAT pathway, astrocytes, neuronal, down syndrome

P7-121

BnH-015B Improves impariments in synaptic plasticity and cognitive behavior in APP/PS1 mice

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Alzheimer's disease (AD) is the most frequent common neurodegenerative disorder that causes dementia in the elderly. Recent evidence indicates that network abnormalities including hypersynchrony, altered oscillatory rhythmic activity, interneuron dysfunction, and synaptic depression may be key mediators of cognitive decline in AD. Therefore, we observed the novel therapeutics BnH-015B effects through electrophysiological experiments in the hippocampus and cerebral cortex, which are known to be related to cognitive ability.
Ex vivo electrophysiological studies were done to investigate the neuronal function of the hippocampus and barrel cortex in the amyloid precursor protein APP/PS1 transgenic mouse. Extracellular field potentials were recorded from the CA1 region of the hippocampus while stimulating the Schaffer collaterals. In addition, thalamocortical (TC) EPSCs were evoked at 0.1 Hz by ventrobasal (VB) stimulation and accepted as monosynaptic when they exhibited a short and constant latency that did not change with increasing stimulus intensity as previously described (Feldman et al., 1998; Isaac et al., 1997). The memory enhancing potential of the compounds was examined using the active avoidance test and for deterioration of memory processes was used BnH-015B in dose 1 mg/kg. Long-term potentiation in the hippocampus was increased in the BnH-015B compared with the vehicle. Also, TC potency in the barrel cortex was increased in the BnH-015B compared with the vehicle. Furthermore, synapse in excitatory to inhibitory (E/I) balance did not change between the two groups. We observed increase in number of responses escaped and avoided in groups treated with BnH-015B in comparison to vehicle. As a result, the presented data in this study suggest that BnH-015B could be used to treat cognitive deficits in AD patients and animal models.

Keywords: small molecule, Alzheimer's disease, APP/PS1, cognitive behavior

P7-122

Development of small-sized brain organoids for live imaging

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Human brain organoids are grown as well-organized 3D cell aggregates containing differentiated neurons and macroglia from human pluripotent stem cells. Although reproducing the live state of the human brain is the advantage of brain organoid culture, their enormous size and movement of Matrigel-embedded organoids disturb the real-time observation of brain organoids. Therefore, miniaturization of the human brain can be the primary substitute for studying live neural circuits and understanding the pathogenesis of ambiguous human brain diseases.
We used a mesh-like extracellular matrix instead of Matrigel to minimize the agitation and size of the 3D cell aggregates for live imaging. The small aggregates that we produced with the optimized protocol clearly show the brain differentiation markers like the typical brain organoids and the visualization of live neurons with a simple incubation of a neuron-specific fluorescent small molecule in the aggregates with a conventional confocal microscope.

Keywords: human brain organoid, ecm, small organoid, live imaging, neuron

P7-123

Particulate matter perturbates melatonin synthesis and secretion through dysregulation of mitochondrial ROS and Ca^{2+} signaling

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Environmental pollution is associated with detrimental health outcomes and increased mortality rates across various diseases. Particulate matter (PM) presents in air pollution has been linked to neurotoxic effects, thereby increasing susceptibility to neurodegenerative diseases and sleep disturbances. Melatonin, a crucial regulator of the sleep-wake cycle, plays a pivotal role in multiple diseases, including impaired immune function and sleep disorders. While previous studies have demonstrated the influence of PM on melatonin secretion from pinealocytes, the underlying mechanism remains poorly understood. In this study, we aimed to investigate the pathological effects of PM on mitochondrial function, specifically its impact on cytosolic Ca^{2+} homeostasis and immune response. Our findings reveal that PM exposure disrupts mitochondrial morphodynamics in pinealocytes through the generation of mitochondrial ROS. These observations suggest that PM-induced mitochondrial dysfunction contributes to a decrease in melatonin synthesis. Additionally, this mitochondrial dysfunction has been found to be associated with the activity of voltage-gated calcium channels (VGCCs), which are involved in the melatonin synthesis and secretion. Significantly, intrathecal injection of PM aggravates the release of TNFα by astrocytes and microglia, subsequently inhibiting melatonin synthesis. Together, our data provide valuable insights into the pathophysiological mechanism through which PM induces mitochondrial dysfunction, leading to the inhibition of VGCC and subsequent disruption of melatonin synthesis. By shedding lights on the intricate interplay between PM exposure, mitochondrial dysfunction, and melatonin regulation, this study contributes to a better understanding of the health implications associated with environmental pollution. These findings underscore the need for effective interventions to mitigate the adverse effects of PM on human health, particularly in relation to sleep disorders.

Keywords: particulate matter, mitochondria, pineal gland, Ca^{2+} signaling

P7-124

Inhibitory effects of monosodium iodoacetate (MIA)-induced low back pain model after pulsed radiofrequency application

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Background: Low back pain is one of most common musculoskeletal disorder. Approximately 70% of people will experience lower back pain (LBP) at some point in their lives, and around 40% of LBP cases can be attributed to degeneration of the intervertebral discs (IVDs). Degenerative changes in the intervertebral discs involve modifications in the organized structure of the disc, along with heightened expression of cytokines, the formation of new blood vessels, and the inducing pain. The low back pain accompanied by these degenerative changes has been observed in previous studies using a disc model where monosodium iodoacetate (MIA) was injected. Various approaches have been attempted to alleviate such low back pain, including not only invasive methods but also physical therapies utilizing various physical factors and manual therapies. However, the factors influencing the effectiveness of pulsed radiofrequency therapy for low back pain have not been known. The objective of this study is to confirm the reduction of low back pain and cytokines by applying pulsed radiofrequency therapy after inducing degenerative disc conditions by injecting MIA into the discs.
Methods: MIA was injected into the disc space (L4-5 and L5-6) of rats. Their behaviors were examined by measuring weight shifts, rearing, and von-Frey tests. We investigated COX-2 and NF-κB in IVD by western blotting. Bone changes were assessed by Micro-CT, and IVD/cartilage changes were measured by hematoxylin and eosin and safranin-o staining and inducible nitric oxide synthase (iNOS) immunohistochemistry. Pulsed radiofrequency therapy is applied for 20 minutes to a pain-induced model created by MIA injection, and the reduction of pain and inflammation cytokines in the disc tissue are subsequently assessed.
Results: We observed a shift in weight bearing to the forefoot and decreased in rearing in MIA-injected rats. Increased NF-κB and COX-2 expression was observed in both the intervertebral disc (IVD) and the left/right dorsal root ganglions (DRGs), while micro-CT analysis indicated a gradual bone deformity in MIA-injected rats. The pulsed radiofrequency group confirmed a reduction in pain and also observed a decrease in COX-2 and IL-1β in the disc tissue.
Conclusion: These results suggest that PRF application improved IVD pain-related behaviors and decreased inflammatory mediator expression in the MIA-induced IVD degeneration rats. It implies that PRF application can serve as a potential therapeutic treatment to relieve LBP with degenerated IVDs.

Keywords: low back pain, monosodium iodoacetate, pain behavior, disc degeneration, pulsed radiofrequency

P8-1

Tools to develop and evaluate of amino acid transport inhibitors

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The identification of high-affinity selective inhibitors for amino acid transporters requires optimization of culture conditions and cell line models to achieve optimal expression of heterologous and endogenous transporters. As a next step, isolation of the transport activity is important to achieve a robust and selective signal. This can be achieved by the addition of specific inhibitors to suppress background activity and by the selection of substrates that are preferred or selective for the transporter under investigation.
We developed several methods to measure amino acid transport in cells, namely a fluorescent membrane potential sensitive assay (FLIPR) for electrogenic transporters, an LC-MS assay and optimised radiolabelled uptake assays.
Radiolabelled uptake assay conditions were optimised for the detection and expression of amino acid transporters SLC6A19 and SLC38A2. This included optimisation of the transport substrate and concentration,

namely isoleucine for SLC6A19 and proline for SLC38A2. Further optimisation included the addition of SLC7A5 inhibitor JPH203 to suppress cell endogenous transport of isoleucine.
We used the LC-MS method to study the activity of amino acid transporters in complex matrices and were able to measure the fluxes of all amino acids simultaneously by using the exchange of 12C and 13C amino acids.
For the FLIPR assay we improved cell culture conditions, cell preincubation and transport substrate to optimise the transport signal.
This transporter toolbox was used for primary high-throughput screening (HTS) and secondary screening of initial hits. The FLIPR assay was the preferred method for HTS, while radiolabelled and LC-MS transport assays were used for secondary screening. This step is important because FLIPR can result in the identification of false-positive hits. Further improvements of initial hits are achieved through medicinal chemistry and structural studies of the target protein including docking and molecular dynamics.
Conclusion: We have developed a toolbox that can be used to identify and characterise transporter inhibitors, which bind to their targets with high affinity and selectivity.

Keywords: SLC6A19, SLC38A2, diabetes, cancer

P8-2

Role of actin in time-dependent shift of docking dynamics of glucagon-like peptide-1 granules during biphasic exocytosis

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Background: Glucagon-like peptide-1 (GLP-1) is an incretin hormone which is secreted by enteroendocrine L-cells in the intestine. GLP-1 secretion is induced by a variety of nutrients. These include glucose, amino acids, and lipids. GLP-1 is secreted by exocytosis of GLP-1-containing granules, which consists of docking, priming, and fusion with the plasma membrane. These docking dynamics are regulated in a variety of cells by intracellular second messengers, such as Ca^{2+} and cAMP, and by the cytoskeleton. However, the temporal regulatory mechanisms underlying the docking dynamics remain unclear. In the present study, we investigated the relationship between intracellular Ca^{2+} and cAMP, and actin with GLP-1 exocytosis.
Methods: We performed live-cell imaging using the mouse enteroendocrine L-cell line GLUTag cells. We used either deoxycholic acid (DCA), which increases the intracellular concentration of both Ca^{2+} and cAMP, or high K^+, which increases only Ca^{2+}. We monitored intracellular Ca^{2+} dynamics with the Ca^{2+}-sensitive dye Fluo4 and cAMP dynamics with the fluorescent protein-based cAMP indicator Pink Flamindo. We also visualized individual exocytosis of GLP-1 granules by total internal reflection fluorescence microscopy. We classified each individual exocytosis into three categories: Old Face (granules are predocked before stimulation and finally fused after stimulation), Resting Newcomer (granules are recruited to the plasma membrane after stimulation and fused after stable docking), and Restless Newcomer (granules are recruited to the plasma membrane after stimulation and fused without stable docking).
Results: Elevation of intracellular Ca^{2+} and cAMP concentration by DCA was mediated by ryanodine receptors and Gs signaling pathway, respectively. Elevation of intracellular Ca^{2+} by high K^+ was mediated by influx of extracellular Ca^{2+}. GLP-1 exocytotic dynamics showed a biphasic pattern, with the first and second peaks of secretion occurring at approximately 8 and 15 minutes after stimulation, respectively. Most exocytotic events were Old Face or Restless Newcomer in the first phase, and Resting Newcomer was dominant in the second phase under either DCA or high K^+. Inhibition of actin polymerization by application of latrunculin A with DCA decreased the proportion of Old Face and increased the proportion of Resting Newcomer.
Conclusion: The results suggest that GLP-1 exocytosis undergoes a time-dependent shift in its docking dynamics, which is regulated by F-actin.

Keywords: exocytosis, glucagon-like peptide-1, second messenger, actin, live-cell imaging

P8-3

Physiological role of the cannabinoid-sensing G-protein coupled receptor 55 in the regulation of ZO-1- and occludin-dependent tight junction integrity and its underlying mechanism

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Background: The orphan G-protein coupled receptor 55 (GPR55) has recently been identified as a cannabinoid receptor that is highly abundant in small intestine with ambiguous function. This study is aimed to scrutinize the physiological role of GPR55 on tight junction integrity in intestinal epithelia, a therapeutic target for intestinal inflammation, since cannabis had been used for the treatment of colitis with unknown mechanism of action.
Methods: T84 monolayers were used as an *in vitro* model of human intestinal epithelia. Minimum Essential Medium Eagle Spinner Modification (S-MEM), a Ca^{2+}-free medium, was used to disrupt intestinal tight junction in T84 monolayers. Measurement of transepithelial electrical resistance (TER) was used to evaluate intestinal tight junction integrity using epithelial Volt/Ohms' meter (EVOM). Fluorescein isothiocyanate–dextran (FITC-dextran) flux assay was used to distinguish paracellular permeability pathway. Specific agonist of GPR55, O1602, was treated in T84 cell monolayers to mimic the physiological response of GPR55. Western blot analysis was performed to identify the related intracellular signaling. In addition, immunofluorescence staining was used to visualize organization of zonula occludens-1 (ZO-1) and occludin.
Results: TER measurement and FITC-dextran flux assay indicated that O1602 treatment enhanced TER value and diminished paracellular permeability across T84 cell monolayers, suggesting that activation of GPR55 increased intestinal barrier function by suppressing leak pathway permeability. Immunofluorescence staining revealed that O1602 treatment dominantly re-organized the anastomosed network of ZO-1 and occludin in T84 cells after being disrupted by Ca^{2+} depletion. Of particular interest, effects of O1602 on intestinal barrier function improvement and tight junction re-organization were abolished by pretreatment with inhibitors of Ca^{2+}/calmodulin kinase kinase beta (CaMKKβ), AMP-activated protein kinase (AMPK), sirtuin-1 (SIRT-1), and extracellular signal-regulated kinase (ERK). Consistently, western blot analysis indicated that O1602 enhanced AMPK and ERK phosphorylation.
Conclusion: These results strongly suggested that activation of GPR55 can promote intestinal tight junction assembly by enhancing ZO-1 and occludin re-organization via CaMKKβ/AMPK/SIRT-1/ERK-dependent mechanism.

Keywords: tight junction, G-protein coupled receptor 55 (GPR55), intestinal barrier function, colitis, cannabis

Poster

Poster

P8-4

Inhibition of intestinal calcium transport by tetrodotoxin and vasoactive intestinal peptide receptor antagonist: An evidence for the neural regulation of intestinal calcium absorption

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The transepithelial calcium transport across the intestinal mucosa is essential for providing calcium for bone formation and several body functions, such as blood coagulation, neurotransmission, cardiac contraction, etc. Under normal conditions, the intestinal calcium absorption is tightly regulated by calciotropic hormones, particularly 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$]. However, it is unclear whether the neural regulation of calcium absorption by enteric neurons or extrinsic autonomic nerves really exists. Since neurons often require voltage-gated sodium channels (Na_v) for generating action potential, blockade of Na_v channels was hypothesized to inhibit neuronal functions. Here, we used tetrodotoxin, which is a potent inhibitor of several Na_v channels such as $Na_v1.1$, $Na_v1.2$, $Na_v1.3$, to demonstrate a contribution of enteric neurons in regulating calcium transport. In an intestinal epithelium-like Caco-2 monolayer cultured on a permeable Snapwell, an exposure to 10 nM 1,25$(OH)_2D_3$ for 72 h markedly increased transepithelial calcium fluxes, as determined by radioactive ^{45}Ca tracer in Ussing chamber. The 1,25$(OH)_2D_3$-treated Caco-2 monolayer probably enhanced the electrogenic ion transport as indicated by an increase in short-circuit current and a decrease in transepithelial resistance. As expected, since Caco-2 monolayer contained no excitable cells, 100 nM tetrodotoxin did affect neither calcium flux nor electrical parameters of the monolayer. On the other hand, a study in the rat duodenum with enteric neurons in the wall showed that 100 nM tetrodotoxin significantly diminished the leucine-induced calcium transport, suggesting the presence of neural regulation. Since vasoactive intestinal peptide (VIP) is an abundant neuropeptide in the intestine where it is capable of modulating ion transport, we used a recombinant inhibitory peptide for VIP receptor to demonstrate whether VIP contributed to calcium transport. The results showed that inhibition of VIP receptor could diminish the 1,25$(OH)_2D_3$-enhanced calcium transport across Caco-2 monolayer. In conclusion, calcium uptake in the rat duodenum, but not Caco-2 monolayer, was found to depend on tetrodotoxin-sensitive Na_v channels, which were abundantly expressed in the enteric neurons. Moreover, the neuropeptide VIP was probably an essential regulator of calcium transport, thereby corroborating the presence of neural regulation of intestinal calcium absorption.

Keywords: calcium transport, duodenum, enteric nervous system, tetrodotoxin, vasoactive intestinal peptide (VIP)

P8-5

Prolonged consumption of high-salt diet alters protein expression levels of TRPV6 and PMCA1b in the villous tips of rat duodenums

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Long-term consumption of diets with high amount of sodium salts is able to disturb the effective circulating volume, thereby increasing susceptibility to hypertension. Previously, we have shown that high-salt diet (HSD) led to negative calcium balance with fecal and urinary calcium wasting as well as an increased cortical bone porosity and osteoclastic bone loss. We therefore hypothesized that the small intestine probably had compensatory responses to help restrict negative calcium balance before fracture occurred. Herein, male Sprague-Dawley rats were treated for 5 months with HSD containing 8% sodium chloride, and the protein expression levels were determined in *ex vivo* duodenal tissues by using Leica SP8 DIVE multiphoton microscope. The results showed that 5-month HSD consumption markedly elevated the daily sodium intake by ~3-fold, thereby increasing fecal sodium output, urinary sodium excretion and serum osmolality, while having no effect on serum sodium levels. Since the intestinal calcium absorption normally requires transient receptor potential vanilloid subfamily member 6 (TRPV6) calcium channels for the apical calcium uptake and plasma membrane Ca-ATPase subtype 1b ($PMCA_{1b}$) for the basolateral calcium extrusion into the extracellular fluid, we quantified the expression levels of both proteins in the duodenal villous tips, where the transcellular calcium transport takes place. A multiphoton immunofluorescent analysis revealed that the protein expression levels of both TRPV6 and $PMCA_{1b}$ were significantly upregulated in the duodenums of HSD rats as compared to those of normal diet-fed rats. It could be concluded that prolonged consumption of HSD was able to increase the duodenal TRPV6 and $PMCA_{1b}$ protein expression, which might be a compensatory response to negative calcium balance.

Keywords: hight-salt diet, intestinal calcium transport, plasma membrane calcium ATPase subtype 1b (PMCA1b), transient receptor potential vanilloid subfamily member 6 (TRPV6)

P9-1

Single and mixed strains of probiotics reduced hepatic fat accumulation and inflammation, and altered gut microbiome in a nonalcoholic steatohepatitis rat model

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Background: As gut dysbiosis has been implicated in the pathogenesis of nonalcoholic steatohepatitis (NASH), probiotic supplement might be a potential treatment for this condition. The aim of this study was to evaluate the effects of mixed and single-strain probiotics on the severity of NASH induced by a high fat, high fructose (HFHF) diet and their gut microbiome related mechanisms.
Methods: Male Sprague-Dawley rats were divided into 4 groups (n=7 per group): Control group, NASH group, NASH + single-strain group and NASH + mixed-strain group. In single-strain and mixed-strain groups, rats received *Lactobacillus plantarum* B7 and *Lactobacillus rhamnosus* L34 + *Lactobacillus paracasei* B13 by oral gavage once daily, respectively. The duration of the study was 6 weeks. Liver tissue was used for the evaluation of histopathology, hepatic fat content by Oil Red O staining, and hepatic TLR-4 and CD14 expression by immunohistochemistry. Fresh feces were collected for gut microbiota analysis.
Results: A higher degree of fat accumulation, hepatocyte ballooning and lobular inflammation were observed in the NASH group as compared with the control group. The amounts of hepatic fat droplets were more pronounced in the NASH group than in the control and treatment groups. Serum TNF-α levels were significantly higher in the NASH group than in control, single-, and mixed-strain groups (274.20±4.68 vs. 44.67±1.64 vs. 44.92±1.73 vs. 49.27±4.21 pg/mL, respectively, $p<0.001$). CD14 and TLR4 expression significantly increased in the NASH group as compared with the control group and decreased in both treatment groups (CD14 positivity, 14.00±3.00 vs. 60.00±9.00 vs. 18.00±1.00 vs. 29.00±5.00; TLR-4 positivity, 5.00±2.00 vs. 45.00±4.00 vs. 16.00±1.00 vs. 20.00±2.00 in control, NASH, single-strain, and mixed-strain groups, respectively). Gut microbiota analyses showed that the alpha diversity was reduced in the NASH group as compared with the control group and improved after both types of probiotic supplement. At the phylum level, the relative abundance of *Proteobacteria* and *Verrucomicrobia* significantly increased in the NASH group compared with the control group. The relative abundance of *Firmicutes* and *Bacteroidetes* increased in both treatment groups and the relative abundance of *Fusobacteria* significantly increased in the single-strain group as compared with the NASH group.
Conclusion: Both single and mixed-strain probiotics could improve NASH pathology through the suppression of TLR4 and CD14 expression and the modification of gut microbiota.

Keywords: probiotics, nonalcoholic steatohepatitis, gut microbiota, TLR4, CD14

P9-2

Oral administration of antioxidant modulated intestinal physiological balance

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Background: For decades, excessive reactive oxygen species (ROS) production has been linked to disease. With the discovery of NADPH oxidases (NOX), a family of enzymes dedicated to the production of reactive oxygen species (ROS) in a variety of cells and tissues under physiological conditions (including intestinal epithelium and phagocyte), this assumption has been revised in recent years. The intestinal epithelial cells express the superoxide- and hydrogen peroxide-producing enzymes NOX1 and DUOX2. According to a recent study, iNOS- and NOX1-dependent ROS production in the ileum of rodents maintains bacterial homeostasis. The surface of the small intestine is the initial site of contact and processing for food. Mucosal oxidative equilibrium could be altered by their exposure to antioxidants derived from food. This study will investigate the role of intestinal reactive oxygen species (ROS) in response to enteral oxidants in mice fed a normal diet.
Methods: C57BL/6 mice, aged 5 weeks and fed a standard diet, are administered enteral antioxidants (vitamin C and tannic acid) in drinking water for 28 days. ROS in the small intestine were measured using L-012-based IVIS imaging on days 7, 21, and 28. At the 28th day, intestinal samples were collected for myeloperoxidase (MPO) and disaccharidase activity assays, and various intestinal segments' mucosal-associated bacteria were counted.
Results: NOX-derived ROS were reduced in mice given enteral antioxidants (vitamin C and tannic acid) with no changes in intestinal morphology. A significant rise in ileal MPO activity was accompanied by an increase in the total number of bacteria in the proximal colon. However, enteral antioxidant has no effect on the activity of jejunal disaccharidase.
Conclusion: Our findings demonstrated that oral antioxidants cause oxidative imbalance and intestinal physiological dyshomeostasis. The detail mechanism requires further investigation.

Keywords: antioxidant, intestine, homeostasis, microbiome, reactive oxygen species

P9-3

High salt diet induces ROCK signaling augmentation and dysmotility in mouse stomach

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Excessive salt intake is a global health problem, particularly prevalent in developed countries, for all ages, genders and backgrounds. Dietary salt intake has been linked to a high prevalence of hypertension, cardiovascular diseases and renal risks, although the impact on gastric functions has yet to be fully examined. In the course of studying impacts of high salt diet on circulatory systems, we found a deformation of stomach in mice, and evaluated pathophysiological impacts of high salt diet on mouse stomach functions. Mice were fed for 2 or 4 weeks with meal including 8% NaCl, and subjected to characterizing high K^+- and carbachol-induced contraction of smooth muscle strips and protein expression,

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as well as function/structure by ultrasonography and histology. High salt diet (HSD, 2 or 4 weeks) resulted in an extended gastric fundus area (forestomach), compared with mice with normal-salt diet (NSD). The extension of the walls were associated with loss of thickness without any signs of apoptosis. Ultrasonography of gastric antrum of mice under anesthesia revealed an impaired gastric motility in HSD mice. Gastric emptying was also suppressed in HSD mice. The carbachol-induced contraction of the strips of HSD-mouse were more sensitive to ROCK inhibitors, H1152 and SR3677, as well as PKC and PDE antagonists, compared with NSD. The gastric dysmotility in HSD mice was associated with upregulation of ROCK expression. In addition, a collagen deposition was found in the smooth muscle layers in gastric walls, suggesting fibrosis. HSD may cause gastric dysfunctions though the disturbed Ca^{2+} sensitization signaling associated with the structural and functional changes. Supported by research funds from Society for Research on Umami Taste and JSPS KAKENHI #JP19K06573 (to ME). No COI.

Keywords: high salt diet, gastroparesis, smooth muscle, Ca^{2+} sensitization, ROCK

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P9-4

Alteration in neural composition of central pathways regulating cololectal motility in parkinson's disease model in rats

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Parkinson's disease (PD) is a neurodegenerative disorder defined by the reduction of dopaminergic innervation in the substantia nigra pars compacta (SNc). PD exhibits a variety of motor and non-motor symptoms. Constipation is one of the prominent non-motor symptoms of PD. We hypothesized that alteration in the central pathways regulating colorectal motility may cause dysmotility of the colon, leading to constipation in PD. The aim of this study was to examine the effects of destruction of dopaminergic neurons in the SNc on composition of the descending neurons that innervated to the lumbosacral defecation center from the hypothalamic A11 region and the medullary raphe nuclei in rats. To destroy dopaminergic neurons in the SNc, 6-hydroxydopamine (6-OHDA) was injected into the right medial forebrain bundle (MFB). As a control group, sham rats were used in which saline was injected into the right MFB using the same procedures. The accuracy of the method for destroying dopaminergic neurons was confirmed by apomorphine-induced rotational movements. Colorectal motility was measured by in vivo methods in anesthetized rats. The colorectum was cannulated at the distal colon and the anus, and filled with saline from a Mariotte bottle connected to the cannula of the distal colon. Intraluminal pressure was measured by a pressure transducer connected to the anal cannula, and the expelled saline from the anus was measured by using a force transducer. In sham rats, intracolonic application of capsaicin as a noxious stimulus enhanced colorectal motility. In contrast, capsaicin failed to enhance colorectal motility in PD rats 3 weeks after 6-OHDA injection. Administration of dopamine or serotonin to the spinal defecation center (L6-S1) in PD rats caused enhancement of colorectal motility, suggesting that stimulatory monoaminergic transmission in lumbosacral spinal cord remains functional in PD rats. When $GABA_A$ receptor inhibitor, bicuculline, was pre-administered to L6-S1 spinal cord, capsaicin enhanced colorectal motility. The noxious stimulus-induced colorectal motility in the presence of $GABA_A$ receptor inhibitor was abolished by pre-administration of a D2-like receptor inhibitor, haloperidol, but not the 5-HT2 and 5-HT3 receptor inhibitors, ketanserin and dolasetron, into the spinal cord. These findings suggest that the disruption of dopaminergic neurons in the SNc alters neural composition of the descending pathways related to regulation of colorectal motility; the serotonergic neurons become inoperative whereas the GABAergic neurons become functional in PD rats.

Keywords: spinal defecation center, dopamine, serotonin, GABA, Parkinson's disease

P9-5

Neural pathways of the dorsomedial hypothalamus activation-induced enhancing colorectal motility

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The mechanism by which acute stress elicits defecation reflex is unknown. It has been reported that a neural pathway from the prefrontal cortical area to the dorsomedial hypothalamus (DMH) is related to thermogenic and cardiovascular responses to stress.
The primary purpose of this study was to verify whether the DMH is involved in defecation reflex pathway. The secondary purpose was to determine neural pathways through which the DMH controls colorectal motility.
Rats were anesthetized with urethane, and colorectal intraluminal pressure and expelled fluid volume were recorded in vivo. we used the chemogenetic technology DREADD (designer receptors exclusively activated by designer drugs) to manipulate activity of specific neurons. To express an inhibitory DREADD, hM4Di, specifically in neurons of the DMH that send the A11 region or the medullary raphe nuclei, the double virus vector infection method, in which two different adeno-associated virus (AAV) vectors are injected into two monosynaptically connected areas, was used.
When AMPA agonist, (S)-AMPA, were unilaterally administered to the DMH, a marked increase in colorectal intraluminal pressure that was associated with increased expelled fluid, in addition to increases in arterial pressure and heart rate, was observed. The enhanced colorectal motility in response to the DMH stimulation was suppressed by prior administration of serotonergic and dopaminergic inhibitors into the spinal cord. The enhanced colorectal motility in response to the DMH stimulation was suppressed by specific suppression of the DMH → medullary raphe pathway or the DMH → A11 pathway.
Our findings that activation of the DMH enhances colorectal motility suggests that the DMH is a part of a neural pathways in stress-induced defecation. It has been known that the medullary raphe nucleus and the A11 nucleus are the origins of serotonergic and dopaminergic descending neurons projecting to the spinal defecation center, respectively. Therefore, our findings suggest that the stimulation of the DMH enhances colorectal motility by activating the DMH → medullary raphe pathway and the DMH → A11 pathway then activating descending neurons projecting to the spinal defecation center. These pathways may cause stress-induced defecation.

Keywords: dorsomedial hypothalamus, colorectal motility, medullary raphe, A11 region, DREADD (designer receptors exclusively activated by

designer drugs)

P9-6

PMCA4 inhibits the activity of nNOS to maintain pacemaker function of colonic ICC

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Background: Interstitial cells of Cajal (ICCs) generate pacemaker currents and are responsible for slow wave generation in gastrointestinal (GI) smooth muscle. Nitric oxide (NO) is one of the most important regulators of GI smooth muscle relaxation. Neuronal nitric oxide synthase (nNOS), which convert the L-arginine to NO and L-citrulline, has been observed in colonic ICC. Overactivity of this enzyme produces excess NO, which inhibits pacemaker currents in the colonic ICC. Plasma membrane calcium ATPase 4 (PMCA4) is not only involved in maintaining calcium homeostasis, but intracellular C-terminal tail also possesses PDZ-domains recognizing amino acid sequence, which can be interacted with nNOS. This study aims to investigate whether PMCA4 can inhibit the activity of nNOS, thereby maintaining the normal pacemaker function of colonic ICC.
Methods: We obtained primary cultured colonic ICCs from ICR mice. RT-PCR, immunofluorescent staining of colon paraffin sections and cultured ICCs, and whole-cell patch-clamp techniques were used in our study.
Results: mRNA transcripts for PMCA (all 1-4 subtypes) and nNOS were expressed in the colonic cells. PMCA4 and nNOS were detected in anoctamin-1 positive cells (ANO1, a specific biomarker for ICC). Aurintricarboxylic acid (ATA, a PMCA4 specific inhibitor) decreased the frequency of pacemaker potentials and slightly hyperpolarized the resting membrane potential in colonic ICC, suggesting that PMCA4 plays an important role in the generation of ICC pacemaker potentials. The effect of ATA was influenced by N_{ω}-Nitro-L-arginine (L-NNA, a nNOS inhibitor) and 1H-[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, a guanylate cyclase inhibitor). These indicates that nNOS and guanylate cyclase activities increase when PMCA4 activity is inhibited.
Conclusion: These results suggest that PMCA4 inhibits the activity of nNOS, thereby avoiding excessive NO production and maintaining normal pacemaker activity of ICC.

Keywords: interstitial cells of Cajal, pacemaker activity, neuronal nitric oxide synthase, plasma membrane calcium ATPase 4, nitric oxide

P9-7

The tight junction protein ZO-1 regulates mitotic spindle orientation to promote mucosal repair in A YBX3 and cytoskeletal binding protein dependent manner

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Damage to the gastrointestinal system may result from physical, infectious, or immune-mediated causes. A rapid repair process, which is crucial for mucosal homeostasis, requires integration of epithelial proliferation, differentiation, and migration. The restoration of the barrier is essential to the recovery process. Although tight junction protein ZO-1 is known to regulate barrier function, its role in maintaining mucosal homeostasis has not been established.
Aim: To determine how ZO-1 regulates mitotic spindle orientation contributing to mucosal repair.
Methods: Public Gene Expression Omnibus data sets and biopsy specimens from patients with inflammatory bowel disease (IBD) and healthy control individuals were analyzed. *Tjp1*$^{f/f}$;vil-Cre^{Tg} mice with intestinal epithelial-specific ZO-1 knockout (ZO-1 KO^{IEC}) mice and *Tjp1*$^{f/f}$ mice littermates without Cre expression were studied using chemical and immune-mediated mucosal injury models as well as intestinal organoid cultures and cell line Caco-2 were used for dissecting the detailed mechanism.
Results: Transcript levels and protein expression of ZO-1 in intestinal epithelia from inflammatory bowel disease (IBD) patients are reduced compared to healthy controls. To determine whether loss of ZO-1 contributes to disease pathogenesis, dextran sulfate sodium (DSS) was administered to ZO-1 KO^{IEC} and *Tjp1*$^{f/f}$ mice. When compared to *Tjp1*$^{f/f}$ mice, ZO-1 KO^{IEC} mice displayed significantly more pronounced mucosal damage and weight loss, and were unable to fully recover. The numbers of Ki67^{+} proliferative cells were significantly reduced in ZO-1 KO^{IEC} mice relative to *Tjp1*$^{f/f}$ mice. In vitro cultures of colonoids derived from *Tjp1*$^{f/f}$;vil-CreERT2^{Tg} mice treated with 4-OHT, which induces ZO-1 deficiency, were smaller and had fewer buds (crypt domains) than those derived from *Tjp1*$^{f/f}$ mice. Anoikis was observed in ZO-1 KO^{IEC} colonoids due to misoriented mitotic spindles that caused one daughter cell to lose contact with the extracellular matrix during mitosis. Live imaging of colonoids from mRFP1-ZO-1 transgenic mice revealed transient accumulation of ZO-1 at the spindle pole, suggesting that ZO-1 interactions at this site are necessary for mitosis to occur effectively. The dividing orientation of Caco-2 cells was not affected by either latrunculin A (lat A) or cytochalasin B (cyto B), suggesting that ZO-1-cortical actin interaction might not be involved in mitotic orientation. ZO-1 deficient cells showed affected levels of cytoskeletal binding proteins and transcription factor YBX3. The results recapitulated the same consequences in IBD patients. Mitosis completion may be facilitated by ZO-1 through cytoskeletal binding proteins, but not by cortical actin.
Conclusion: ZO-1 plays an important, although underappreciated, role in the orientation of mitotic spindles. It is hypothesized that YBX3 and ZO-1-associated cytoskeletal binding proteins control mitotic orientation. This may explain the ineffective healing of mucosa in patients with IBD.

Keywords: tight junction, mucosal repair, epithelial homeostasis, mitotic spindle, inflammatory bowel disease

P9-8

Effect of *Atractylodes macrocephala Koidzumi* on intestinal function, intestinal microbiome and ion channels in a mouse model of Zymozan-induced irritable bowel syndrome

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Irritable Bowel Syndrome (IBS) is a common functional gastrointestinal disorder that is associated with abdominal pain, discomfort, and altered bowel movements. The cause of IBS, characterized by dysfunction without anatomical abnormalities in colon, is not well known. *Atractylodes*

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macrocephala Koidzumi (AMK) is a traditional herbal medicine widely used in East Asia for the treatment of digestive system diseases. We investigated the effect and related mechanism of AMK on the treatment of IBS. First, we investigated the efficacy of AMK in the zymosan-induced IBS animal model. The IBS model was established by intracolonic administration of zymosan. Also, to investigate the relevant mechanisms, we used electrophysiological methods to determine the modulation of transient receptor potential (TRP) and voltage-switched Na^+ (NaV) ion channels. As a result, AMK oral administration showed increased colon length and decreased stool score and colon weight compared to the control group. In addition, AMK controlled weight loss without significantly affecting food intake. In the AMK administration group, mucosal thickness was reduced, similar to normal mice, and pain-related behavior was significantly reduced. These effects were similar to those of sulfasalazine, an anti-inflammatory drug, and amitriptyline, an antidepressant. AMK inhibited TRPV1, NaV1.5 and NaV1.7 ion channels associated with IBS-mediated visceral hypersensitivity. These results suggest that AMK could be a potential therapeutic agent for IBS through its modulation of ion channels involved in pain and inflammation.

Keywords: atractylodes macrocephala Koidzumi (AMK), irritable bowel syndrome, gastrointestinal disorders, Zymosan, ion channels

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P9-9

Synbiotic mixture migrates stress-induced intestinal morphology abnormalities and gut leakage through restoration of ZO-1 protein expression

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The impact of stress on intestinal epithelial permeability and the subsequent development of intestinal leakage disorders have been well-concluded. However, the effects of stress induction on the gut microbiome remain unclear. Synbiotics (Syn), a combination of probiotics and prebiotics, have demonstrated the ability to modify the gut microbiome.
Therefore, the aim of this study was to investigate the efficacy of Syn in reducing stress-induced alterations in ileum morphology, intestinal permeability, and the expression of tight junction proteins. Twenty-four male adult Wistar rats were divided into three groups (8 rats/group): control, stress + vehicle, and stress + Syn. The stressed rats were immobilized in a restrainer for 2 hours each day over a period of 14 days and orally administered a Syn mixture containing $1.0x10^{10}$ CFU *Bifidobacterium*, *Lactobacillus* strains, and oligosaccharides, while the control and stress + vehicle groups received normal saline (5 mL/kg). Ileum histology, fluorescein isothiocyanate-dextran (40 kDa FITC-Dextran) permeability, and the expression of ZO-1 protein were evaluated.
The results demonstrated that immobilized-stressed rats exhibited stress-induced reductions in villi/crypt ratio as well as increased intestinal permeability compared to the control group. Furthermore, the stress + vehicle group showed downregulation of ZO-1 protein expression in the ileum. However, Syn supplementation effectively attenuated these stress-induced changes by improving the integrity of the ileum and restoring the expression of tight junction proteins in the stressed rats.
In conclusion, the supplementation of this Syn formulation showed promise in mitigating stress-induced alterations in intestinal morphology abnormalities and gut leakage. These findings highlight the potential of Synbiotics as a therapeutic intervention, providing valuable insights into the mechanisms by which Syn modulates gut barrier function and improves intestinal health in stressed individuals.

Keywords: intestinal permeability, restraint stress, synbiotics, villi/crypt ratio, ZO-1

P9-10

Preliminary efficacy analysis of *Streptococcus thermophilus* iHA318 in alleviating dry eye symptom

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Dry Eye Syndrome refers to a condition with insufficient tear production, uneven tear distribution, or excessive evaporation of tears, leading to inadequate moisture on the eye surface. This study aimed to elucidate the efficacy of a probiotic strain, *S. thermophilus* iHA318 to alleviate dry eye symptoms by using a 6-week-old female ICR mouse model induced by UVB irradiation. This study is conducted in collaboration with Percheron Bioceutical Co. Ltd. The investigation was designed as a short-term study (11 days), and the mice were allocated into 5 groups (n=3 in each group) as follows: (1) Control group: 0.9% NaCl solution, (2) Damage group: 0.9% NaCl and damaged by UVB, (3) Probiotics medium-dose group: iHA318 at a medium dose and damaged by UVB, (4) Probiotics high-dose group: iHA318 at a high dose and damaged by UVB, (5) Artificial tears group: 0.9% NaCl with an application of artificial tears and damaged by UVB. All the 0.9% NaCl solutions and probiotics were given by oral gavage twice daily, in the morning and the evening, from day -2 to day 7. UVB irradiation was performed from day 1 to day 7 with a daily irradiation dose at 0.72 J/cm^2. Tear volume and tear film break-up time were measured on day 0, 4, and 7. On day 8, the ocular surface status was assessed, followed by the sacrifice of the mice. The ocular tissues from the high-dose group were processed for tissue sectioning and staining. The tear volume showed that the high-dose group was significantly increased compared to the damage group, indicating that a high dose of iHA318 may increase tear secretion. The histological staining results showed that iHA318 could alleviate lacrimal gland damage and promote normal differentiation of corneal epithelial cells and conjunctival goblet cells.

Keywords: dry eye, probiotics, lacrimal gland, short-term effect

P9-11

Sigma-1 receptor agonist PRE084 is protective against acetic acid-induced experimental colitis in mice

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Background: The aim of this study was to investigate the protective ef-

fect of sigma-1 receptor on acetic acid (AA)-induced colitis in mice.
Methods: Twenty-four male ICR were randomly divided into four groups (6 mice/group): a control group, colitis group (chemically induced by the intrarectal administration of 5% AA), PRE084-pretreated group (pretreatment withPRE084, followed by 5% AA), and PRE084 plus BD1047-pretreated group (co-treatment with PRE084 and BD1047, followed by 5% AA). PRE084 and BD1047 were injected daily from 3 days before the onset of colitis to 1 day before the end of experiment. The distal colon was excised for macroscopic and microscopic evaluations and analysis of the levels of tumor necrosis factor-alpha (TNF-α), interleukin g (IL-6), IL-10, glutathione, catalase, superoxide dismutase (SOD), myeloperoxidase (MPO), and lipid peroxidation.
Results: The colitis group exhibited weight loss, mucosal damage, increases in TNF-α, IL-6, MPO, and thiobarbituric acid reactive substance activity, and decreases in IL-10, glutathione, SOD, and catalase activity. These AA-induced changes were significantly attenuated by PRE084 pretreatment. When BD1047 was combined with PRE084 to block the protective effect of PRE084, parameter values were consistent with those in the 5% AA-induced colitis group.
Conclusion: The sigma-1 receptor agonist PRE084 exhibits protective effects against AA-induced colitis by reducing inflammation and oxidative stress and increasing antioxidant properties.

Keywords: acetic acid-induced colitis, PRE084, BD1047, mice

P9-12

Regulation of smooth muscle contraction in mouse ileum by mechanosensitive cationic piezo1 channels expressed in interstitial cells of Cajal

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The contractile activity of gastrointestinal (GI) smooth muscles changes in response to mechanical stretch from luminal contents, possibly due to activation of mechanosensitive ion channels. However, the specific molecular entities and physiological functions of these channels remain to be elucidated. In this study, we investigated the roles of Piezo1 channels, recently identified as a new class of mechanosensitive non-selective cationic channel, in the regulation of small intestinal motility in mice by measuring the effects of the channel activator Yoda1 and inhibitor GsMTx-4 on GI transit rate and ileal smooth muscle contractility. The GI transit rate was measured as the length of the small intestine traversed by a charcoal meal, while longitudinal tension was measured in ileal segments subjected to a basal tension of 0.3 g. Administration of Yoda1 (5 mg/kg) significantly increased GI transit rate in ddY mice, while GsMTx-4 (1 mg/kg) slowed transit rate, although the latter difference did not reach statistical significance compared to controls ($p = 0.059$). Application of Yoda1 (10 μM) augmented the spontaneous contractions whereas GsMTx-4 (10 μM) suppressed the spontaneous contractions of ileal segments from ddY mice. Further, these Yoda1-induced contractions were substantially inhibited by preincubation with the Yoda1 antagonist Dooku1 (10 μM). To investigate the involvement of enteric nerves and interstitial cells of Cajal (ICCs) in Yoda1-induced contractions, the responses were recorded in ileal preparations from ddY mice pretreated with tetrodotoxin (TTX) and in segments from W/W^{v} mutant mice, which harbor an ICC deficiency in the myenteric plexus (ICC-MY). Pretreatment with TTX (1 μM) had little effect on Yoda1-induced contractions in ddY mouse preparations, suggesting that the response is independent of enteric nerve activity. Moreover, Yoda1-induced enhancement of spontaneous ileal contractions was significantly attenuated in preparations from W/W^{v} mice compared to preparations from +/+ control mice. These results suggest that Piezo1 channels expressed in ICC-MY contribute to mouse ileal smooth muscle contraction.
This work was supported by JSPS KAKENHI Grants 20K07093 and 23K06175.

Keywords: Piezo1 channel, mechanosensitive ion channels, smooth muscle cell, interstitial cells of Cajal, contraction

P9-13

Molecular triggering mechanism of hepatocyte regeneration after massive hepatectomy in mice

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Background: To investigate the molecular triggering mechanism of hepatocyte regeneration after massive hepatectomy in mice.
Methods: This study included 49 C57BL/6 wild-type mice that were divided into 7 groups randomly. Group A is normal control. The group B, D and F mice were 1, 3 or 7 days respectively after sham operation. Those mice underwent abdominal operation without hepatectomy. Group C, E and G mice were 1, 3, and 7 days respectively after undergoing abdominal operation with massive hepatectomy. Massive hepatectomy means that the intermediate and left lobes of the liver were resected, and the right lobe of the liver was remained. The skin was then closed with a running 4-0 nylon suture. The weight of the livers were measured at the end of an experimental day. mRNA of VL-30, PSF, and Rab23 were quantified by using the real-time reverse transcription polymerase chain reaction (RT-qPCR). The correlation between the liver weight and mRNA quantities of the genes were analyzed. The VL-30 lncRNA and PSF protein expression in NIH-3T3 cells were double stained simultaneously by in situ hybridization and immunofluorescence technology for learning more details about the hepatocyte regeneration mechanism.
Results: The right robes accounting for 31.5% of the whole liver in group A. After massive hepatectomy, the remaining right robes grew quickly to 45%, 71.6% and 89.1% respectively in group C, E, and G, compared to the whole liver of the mice in the sham operation groups B, D, and F. Compared to the mice in the sham operation groups, lncRNA VL-30 increased significantly at day 1, returned to the normal level at day 3, and decreased at day 7 in the mice of the massive hepatectomy groups. PSF mRNA presented similar pattern with VL-30 lncRNA but milder than VL-30 lncRNA. However, Rab23 mRNA elevated until day 7 in the massive hepatectomy group. Simultaneously double stained VL-30 lncRNA and PSF protein on NIH-3T3 cells presented that VL-30 lncRNA increase much more density in mitosis phase cells than in the non-mitosis cells. PSF protein presented adverse pattern. Moreover, PSF protein existed in nucleus in the non-mitosis cells, and it shift to cytoplasm while cells were undergoing mitosis.
Conclusion: VL-30 lncRNA expression increase, PSF protein expression decrease, and PSF left nucleus [Lenovo1] were the molecular triggering mechanism of hepatocyte regeneration after massive hepatectomy in mice.

Keywords: hepatectomy, hepatocyte regeneration, VL-30 lncRNA, PSF, C57BL/6 mouse

Poster

P9-14

WITHDRAWN

P10-1

Activation of TGR5 increases urine concentration by inducing AQP2 and AQP3 expression in renal medullary collecting ducts

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G protein-coupled bile acid receptor (TGR5), the first G protein-coupled receptor for bile acids identified, is capable of activating a variety of intracellular signaling pathways after interacting with bile acids. TGR5 plays an important role in multiple physiological processes and is considered to be a potential target for the treatment of metabolic diseases including type 2 diabetes. Evidence has been emerged that genetically deletion of TGR5 results in an increase in basal urine output, suggesting that TGR5 may play an important role in renal water and salt reabsorption. The present study aims to elucidate the effect and mechanism of TGR5 activation on urine concentration. Treatment of mice with the TGR5 agonist LCA and INT-777 markedly reduced urine output and increased urine osmolality, accompanied by a marked increase in AQP2 and AQP3 protein expression and membrane translocation. In cultured primary medullary collecting duct cells, LCA and INT-777 dose-dependently upregulated AQP2 and AQP3 expression by cAMP/PKA-dependent pathway. Mechanistically, both AQP2 and AQP3 gene promoter contains a putative CREB biding site, which can be bound and activated by CREB as assessed by gene promoter-driven luciferase and gel shift assays. Collectively, our findings demonstrate that activation of TGR5 can promote urine concentration by upregulation of AQP2 and AQP3 expression in renal collecting ducts. TGR5 may represent an attractive target for the treatment of patients with urine concentration defect.

Keywords: TGR5, bile acid, AQPs, collecting duct, urine concentration

P10-2

Pregnane X receptor alleviates renal fibrosis by inhibiting the activation of Wnt/β-catenin signaling pathway via interacting with p53

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Renal fibrosis is a common pathological feature of chronic kidney disease (CKD), leading to the loss of nephrons and impairment of renal function. Pregnane X receptor (PXR), a member of nuclear receptor superfamily, has been reported with a constitutive renal expression and protective effect in acute kidney injury (AKI). However, the role of PXR in renal fibrosis remains unclear. The aim of this study was to investigate the role and mechanism of PXR in renal fibrosis.

Renal fibrosis model was established in C57BL/6J wild-type mice, PXR gene knockout ($PXR^{-/-}$) mice and humanized PXR (hPXR) mice by both 0.2% adenine diet model and unilateral ureteral obstruction model (UUO). We demonstrated that the activation of PXR by its agonist pregnenolone-16α-carbonitrile (PCN) reduced urinary albumin, serum creatinine and urea nitrogen content, alleviated renal tubular injury and renal interstitial fibrosis in mice induced by 0.2% adenine diet. PCN treatment attenuated adenine-induced upregulation of P-smad3, αSMA, Collagen I and Fibronectin. In addition, the expression of P-smad3 in the nucleus was also inhibited by PCN. We also performed the experiments in UUO model and got the same results. In addition, we treated hPXR mice with rifampicin (RIF) which is a human PXR agonist and found that RIF showed similar therapeutic effect in 0.2% adenine diet model. On the contrary, PXR knockout aggravated renal fibrosis induced by 0.2% adenine diet and UUO. Wnt/β-catenin signaling pathway has been reported to be closely involved in renal fibrosis. We detected the expressions of all Wnt family genes located in the kidney and found that PCN significantly inhibited the expression of Wnt7a in vivo and in vitro. Expression and activation of β-catenin, a key factor of Wnt/β-catenin signaling pathways, was also inhibited by PCN. Double immunofluorescence and Co-IP assays revealed that p53 and PXR co-localized and interacted with each other in mouse renal proximal tubular cell line. ChIP assay indicated that p53 effectively collect the chromatin fragment of Wnt7a promoter which was predicted containing p53 binding site by the PROMO database. PXR activation drastically reduced the binding of p53 in Wnt7a promoter. In addition, dual luciferase analysis showed that p53 overexpression promoted Wnt7a transcription, but this effect was inhibited by PXR activation. Furthermore, overexpression of p53 increased the expression of Wnt7a, β-catenin and their downstream target gene Fibronectin, while Wnt7a knock down abolished this effect. Taken together, our results demonstrated that PXR plays a protective role in renal fibrosis, PXR inhibits Wnt7a/β-catenin signaling pathway by interacting with p53. The present study indicated that PXR might be a new target for clinical treatment of renal fibrosis in the future.

Keywords: renal fibrosis, PXR, Wnt/β-catenin, p53, Wnt7a

P10-3

Role of melatonin on kidney injury and its remote organ consequences on the liver after renal ischemia and reperfusion injury in D-galactose-induced aging rats

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Renal ischemia and reperfusion (RIR) is the leading cause of acute kidney injury that usually progresses to chronic kidney disease and finally end-stage renal disease. RIR and its remote organ consequences remain the major problem in clinical practice. The condition is becoming a more serious concern among the elderly due to its high prevalence and mortality. As the world is now entering an aging society, it is crucial to find effective treatments to improve the quality of life in the elderly. Mitochondria, apart from being a powerhouse of the cell, play a significant role in diverse regulatory mechanisms in the body. Dysfunction of the mitochondria are well recognized as underlying causes of various oxidant-related diseases, including RIR. Melatonin, a hormone produced by the pineal gland, has potent antioxidant and mitochondrial protective properties. It is probable that melatonin can improve RIR-

induced renal damage and hepatic distant organ injury in the elderly. This study aimed at testing this hypothesis in a D-galactose-induced aging rat model. Male Sprague-Dawley rats were divided into 5 groups (n=6 each): Control-Sham group, Control-RIR group, Aged-Sham group, Aged-RIR group, and Aged RIR-Mel group (melatonin 10 mg/kg i.p., 5 min before reperfusion). Aging was accelerated by D-galactose injection (150 mg/kg/day s.c.) for 8 weeks before underwent RIR or sham operation. RIR was induced by clamping both renal pedicles for 45 min followed by reperfusion for 24 hrs. Kidney and liver functions remained unchanged in the Control-Sham and Aged-Sham groups. The Aged-RIR group exhibited severe kidney and liver damages, evidenced by significant increases in serum levels of creatinine, kidney injury molecule-1, aspartate transaminase, and alanine transaminase, compared to the Control-RIR group. Kidney and liver mitochondrial dysfunctions as indicated by mitochondrial swelling, increased ROS production, and membrane potential dissipation were also more severe in the group of Aged-RIR than Control-RIR. Light and electron microscopies provided further support to the observed functional impairments. All the changes caused by RIR were significantly improved in the Aged RIR-Mel group. The study revealed that RIR-induced kidney and remote organ liver injuries are aggravated in aging, and melatonin has potential to serve as a treatment option for this condition through its mitochondrial protection.

Keywords: melatonin, mitochondria, acute kidney injury, D-galactose, aging

P10-4

Correlation between sleep duration and degree of dehydration in young adults

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Background: A survey by Honestdocs in 2019 found that 67% of Indonesians in the age range of 18 to 24 years were sleep-deprived. Young adults tend to sacrifice sleep to do their tasks and fulfill their responsibility in college and the workplace. For them, sleep is a luxury, not a necessity. Sleep deprivation is known to cause various acute and chronic health problems, but not many people know that sleep deprivation also causes dehydration. A study by The Indonesian Regional Hydration Study (THIRST) in Indonesia in 2008 found that 46,1% of Indonesians were minimally dehydrated. Although the prevalence of sleep deprivation and dehydration in Indonesia is high, no Indonesian study has examined whether there is a relationship between sleep deprivation and dehydration. Even though dehydration is an important public health issue that affects the quality of life and can cause long-term complications. Therefore, more study is needed to find the relationship between sleep deprivation and dehydration. The objective of this study is to determine the correlation between sleep duration and degree of dehydration using the Sleep Timing Questionnaire (STQ) and urine dipstick. In this study, we hypothesize that less sleep duration significantly correlates with a higher degree of dehydration.
Methods: This was a cross-sectional study involving young adults ranging from 17 to 22 years old. This study was performed from October to November 2020 at the School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, Jakarta. The study sample was taken by simple random sampling. A total of 98 participants signed the informed consent and were included in the study. The inclusion criteria were active preclinical students who agreed to become research subjects; had a normal Body Mass Index; and drank ≥1,88 L of water/day (women) or ≥2 L of water/day (men). The degree of dehydration was measured using urine specific gravity value in a urine dipstick. Participants were asked to collect a midstream morning urine sample and dip the urine dipstick. Sleep duration was measured by Sleep Timing Questionnaire (STQ). The data were analyzed using the Spearman correlation test in the IBM-SPSS statistics program version 25. P-value was two-tailed and significance was set at $p<0.05$ with a 95% confidence interval.
Results: This study shows that most of the participants slept for <6 hours per night (31.6%) and were minimally dehydrated (78.6%). Most of the male participants slept for <6 hours per night (34.7%), while female participants slept for 7 hours per night (32.7%). Even though both of them were minimally dehydrated, the prevalence of minimal dehydration was higher in female participants (81.6%) than in male participants (75.5%). The bivariate analysis didn't show any correlation between sleep duration and degree of dehydration ($p=0.418$).
Conclusion: Our findings indicate that there was no correlation between sleep duration and the degree of dehydration in young adults.

Keywords: sleep, degree of dehydration, sleep duration, young adults, hydration

P10-5

Apolipoprotein J facilitates indoxyl sulfate-induced activation of AhR and promotes the progression of chronic kidney disease

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Background: Accumulation of indoxyl sulfate (IS) in tubule cells promotes renal pathogenesis and contributes to the progression of chronic kidney disease (CKD). Apolipoprotein J (ApoJ), a molecular chaperone responding to extracellular stress, was found to be accumulated in renal tubule cells and significantly upregulated in patients with CKD, but the underlying mechanism remains elusive.
Methods: ApoJ levels were determined in serum and tissue of mouse models of CKD and the associations between serum ApoJ and IS were validated in patients with CKD. The differential expression proteins in renal proximal tubular epithelial (HK2) cells with/without ApoJ expression were quantified by iTRAQ and analyzed KEGG pathway enrichment analysis. The effect of ApoJ on the IS-induced activation of AhR is validated in vitro and in vivo.
Results: The expression of ApoJ was significantly increased in the serum and renal tissues of mouse of UUO and the positive correlation between serum ApoJ and IS was found in patients with CKD. Functionally, ApoJ promoted nuclear translocation of AhR and facilitated IS-induced renal pathogenesis. ApoJ silencing, on the other hand, relieved EMT and decreased ROS production in IS-treated HK2 cells. Supportively, liver-specific ApoJ knockout reduced serum IS levels, prevented renal accumulation of ApoJ, and improved the disease progression in mice with CKD. Finally, a peptide against ApoJ chaperone activity was proposed as a new therapeutic strategy to block IS-induced AhR activation and inhibit EMT in renal tubule.
Conclusion: Tubule accumulation of ApoJ promotes renal damage through facilitating AhR activation and targeting ApoJ may be a novel approach against CKD.

Keywords: chronic kidney disease, indoxyl sulfate, apolipoprotein J, Aryl hydrocarbon receptor

Poster

P10-6

Apolipoprotein J accumulation in renal tubules contributes to pathogenesis of diabetic nephropathy

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Background: Diabetic nephropathy (DN) is one of the leading causes of end-stage renal disease (ESRD). The pathogenesis of DN is closely related to lipotoxicity caused by lipid deposition in renal tubular cells. Apolipoprotein J (ApoJ) had been identified as a hepatokine and played an important role in intracellular homeostasis. However, the role of ApoJ in the DN pathogenesis remains unclear.
Methods: The blood samples from normal subjects and patients with prediabetes, type-II diabetes, and DN were collected to assess the clinical relevance of ApoJ-associated pathogenesis. The pathways involved in ApoJ-regulated metabolic imbalance were identified by proteomic approaches and validated using *in vitro* and *in vivo* gain or loss function assays. The interaction between ApoJ and client proteins were verified by immunoprecipitation experiments. The ApoJ regulated autophagy-lysosome pathway was dynamically addressed via live imaging and autophagic flux analyses.
Results: ApoJ was found to be significantly increased in patients with DN and closely related to DN pathogenesis. By using animal models, we identified that ApoJ is accumulated in renal tubules and contributes the diseases progression of DN. The mammalian target of rapamycin (mTOR) pathway was enriched by KEGG analysis. Functional experiments next revealed that ApoJ impeded nuclear translocation of transcription factor EB (TFEB) which impairs autophagy and lysosomal function and contributes to intracellular lipid and ROS homeostasis imbalance. Targeting ApoJ chaperone activity, on the other hand, reactivated TFEB. rebalanced metabolic homeostasis, and alleviated DN progression.
Conclusion: Accumulation of ApoJ in renal tubules facilitates DN progression via regulating mTOR-TFEB-autophagy axis. Thus, antagonizing ApoJ chaperone activity represents a potential therapeutic approach for DN.

Keywords: apolipoprotein J, Diabetic nephropathy, translocation of transcription factor EB

P10-7

Evaluation of nephrotoxicity and polycystic kidney disease induced by chronic exposure of ethylenethiourea in mice

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Ethylenethiourea (ETU) is one of the main metabolite of ethylenebisdithiocarbamate fungicides and potential exposure is highest for workers involved in rubber and fungicide production. Exposure of ETU induces endocrine disruption, teratogenesis, carcinogenicity, and goitrogenicity. Recently, it has been reported that high dose of ETU (300 mg/L) resulted in ultrastructure alteration in proximal tubular epithelial cells. In the present study, we evaluated that the changes of visceral organ weight, cholesterol levels in serum, renal and liver function index, and epigenetic miRNA expression levels in C57BL/6 mouse with chronic exposure of ETU for 58 weeks. Chronic exposure of low dose ETU (2 mg/Kg body weight/day) induced toxicological effects which as followed; 1) lowered body weight, 2) increased triglyceride and cholesterol in serum, 3) increased blood urea nitrogen (BUN) and creatine levels, 4) induced extreme malfunction of kidney including decreased number and size of glomerulus, 5) and induced severe hydronephrosis or polycystogenesis compared to the control. Also, ETU diet increased expression levels of miR-1971, miR-155, miR-135, miR-125, and miR-21, as known to biomarker for renal injury and fibrosis, in kidney. In the cause of polycystic kidney disease, ETU diet increased expression levels of miR-17~92 cluster, known as an oncogenic miRNA cluster and renal cyst growth, and miR-182, a novel regulator of actin cytoskeleton and cyst progression. Taken together, these data suggest that chronic exposure to ETU, at low concentration without causing acute toxicity, evoked renal dysfunction such as glomerular dysfunction and renal cyst development.

Keywords: nephrotoxicity, polycystic kidney disease, ethylenethiourea, miR-17~92 cluster, plasma creatine

P10-8

Protective effects of prebiotic fructo-oligosaccharide on renal organic anion transporter 3 and renal function in pre-diabetic rats

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Background: The prevalence of obesity is increasing world-wide due to high-fat diet consumption and it was confirmed to be a promoter of insulin resistance and type 2 diabetes mellitus (T2DM). Obesity is related to adipose tissue dysregulation, systemic-inflammation result in overactivation of kidney inflammation, oxidative stress, fibrosis and apoptosis. Gut dysbiosis, an "imbalance" in the gut microbial community, has been recently linked to the initiation and consequence of metabolic disturbance and its related complications especially kidney injury. Consumption of prebiotics been reported to improve gut dysbiosis and decelerate the progression of kidney disease. However, the underlined mechanisms of prebiotic consumption on the protection of renal dysfunction and the inflammatory signaling in gut and kidney have not been clearly explored. This study aimed to examine the effects of prebiotic, fructo-oligosaccharide (FOS), on metabolic profiles and kidney function in high-fat diet-induced obese-insulin resistant rats.
Methods: Male Wistar rats were induced by HFD consumption for 16 weeks for developed obese-insulin resistant condition. After that, the HFD fed rats were received FOS l g/day (HFFOS1), or metformin (30 mg/kg/day) (HFMET) which was administered daily by oral gavage for 8 weeks.
Results: The results indicated that FOS could attenuate metabolic disturbance including obese, insulin resistance, improve renal function and decelerate renal inflammation, oxidative stress, and fibrosis, improve renal function, and histopathological changes in HFFOS1 rats. Moreover, we found that FOS has higher efficacy than metformin in the diminishment of intestinal injury induced by HFD.
Conclusions: These data suggested that FOS might be used as a supplement for the therapeutic purpose in an obese condition to prevent renal complication.

Keywords: high-fat diet, insulin resistance, prebiotic, fructo-oligosaccharide, Kidney injury

P10-9

Echinochrome A reverses kidney abnormality and reduces blood pressure in a rat model of preeclampsia

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Background: To explore the effects of Echinochrome A (Ech A) on systemic changes using a rat model of preeclampsia.
Methods: Preeclampsia was induced by infusion of angiotensin II (Ang II) through osmotic pump (1mg/kg/min) on the gestation day(GD) 8. Ech A treatment on GD 14 (100mg/ml) through jugular vein. Observe the systolic and diastolic blood pressure of rats from GD 8 for 2 weeks, determine their fetal weight, fetal crown-rump length and placental weight on harvest day (GD 22). Observe the histological changes of tissues through H&E staining. mRNA expressions of TNF-α, (IL)-10 and VEGF were determined by qRT-PCR, protein expressions of Bcl -2, Bax and Bcl-2/Bax were determined by Western blotting.
Results: The diameters of glomerulus were expanded and capillaries of glomerulus were diminished. No change was observed in the heart and liver in Ang II group but epithelial structure was disrupted in the uterus. Ech A reduced systolic/diastolic blood pressure, reversed glomerulus alterations but did not affect fetal weight, fetal crown-rump length or placental weight. Ech A only partly reversed the effect on uterus. mRNA expressions of TNF-α was increased; (IL)-10 and VEGF were reduced in four tissues tested in Ang II group and Ech A restored these changes in all tissues. Furthermore, Bcl -2 was reduced and Bax was increased, resulted in significant increase in Bcl-2/Bax ratios in Ang II and Ech A reversed these changes.
Conclusion: We suggest that Ech A modulates inflammation and apoptosis in systemic organs in Ang II-induced rat preeclampsia and preserves kidney structure and reduces blood pressure.

Keywords: echinochrome A, TNF–α, angiotensin II, apoptosis

P10-10

Maternal obesity promotes kidney injury in male offspring

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Obesity during pregnancy increases the risk of obesity, diabetes, insulin resistance and the development and progression of chronic kidney disease (CKD) in later life. Impaired renal autophagy is linked to kidney dysfunction in the setting of increased renal lipid accumulation. The aim of this study was to elucidate the effect of maternal obesity on kidney injury related impaired renal autophagic process in the offspring. Female C57BL/6 mice were fed a high-fat diet (HFD) for 8 weeks before breeding and throughout gestation and lactation. Male offspring were selected and examined at weaning. Maternal obesity induced obese insulin resistance and hyperlipidemia and consequently promoted kidney injury in the offspring. Obesity in mother increased lipid transporter in the renal cells, CD36, in the offspring. In addition, FAS, SREBP1 and perilipin-2 were activated while PPARα and CPT1 were suppressed leading to an increase in renal lipid accumulation in the offspring. In light of this, the rising of renal lipid accumulation was related to the impaired autophagic process and lysosomal formation. These results accelerated kidney injury and contributed to an increased susceptibility to CKD in male offspring born to maternal obesity. Therefore, the knowledge gained from this study might be applicable for advancing future preventive approaches for obesity-related kidney injuries and childhood CKD progression.

Keywords: maternal obesity, offspring kidney, kidney injury, renal lipid metabolism, renal autophagy

P10-11

Nephroprotective effects of combined administration of *Nelumbo Nucifera* seed extract and angiotensin-converting enzyme (ACE) inhibitor in L-NAME-induced hypertensive rats: a study on renal structural changes

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High blood pressure provokes kidney injury, leading to remarkable structural alterations such as glomerulosclerosis, tubulointerstitial fibrosis, and tubular atrophy. Due to the limited availability of therapeutic options for kidney protection, there is a continuous pursuit of new strategies aimed at preserving kidney function in hypertension. *Nelumbo* Nucifera, commonly known as lotus, contains seed that are abundant in a variety of substances with antioxidative stress and anti-inflammatory properties. This study sought to assess the nephroprotective effects of lotus seeds extract (LSE) and/or captopril in L-NAME hypertensive rats. Thirty-five adult male Sprague-Dawley rats were allocated into seven groups as follows: the normal control group; the L-NAME group, which received NG-nitro-L-arginine methyl ester (L-NAME) at a dosage of 40 mg/kg/day in tap water to induce hypertension; the LSE group, which received lotus seed extract at dosages of 5, 10, and 100 mg/kg/day; the Cap group, which received captopril at a dosage of 5 mg/kg/day; and the LSE+Cap group, which received a combination of LSE at a dosage of 5 mg/kg/day and captopril at a dosage of 2.5 mg/kg/day. After 35 consecutive days of treatment, the administration of L-NAME significantly increased both the average systolic blood pressure (SBP) by 37.5% and the glomerular tuft area compared to normal rats ($p < 0.05$). Additionally, various kidney injuries were observed in the renal cortex, including glomerular capillary retraction (glomeruli damage score of 2), renal tubular inflammation, necrosis, and loss of brush border (tubular damage score of 3.5). When administered individually, LSE at doses of 10 mg and 100 mg, along with a 5 mg dosage of Cap, effectively reduced the glomerular tuft area compared to the L-NAME group ($p < 0.05$). Notably, the combination treatment involving a low dose of LSE in conjunction with half the dose of captopril demonstrated an absence of glomerular capillary retraction. Instead, it revealed only renal tubular injuries and mild inflammation within the tubulointerstitial. *Nelumbo Nucifera* seed extract exhibited a protective effect against kidney injury resulting from high blood pressure, and its efficacy was further enhanced when used in combination with captopril.

Keywords: Nelumbo Nucifera, lotus seed, high blood pressure, kidney injury, L-NAME

Poster

Poster

P10-12

Protective effects of echinochrome A on Type 2 diabetes-induced nephropathy in db/db mice

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Echinochrome A (EchA) is a naturally occurring substance derived from sea urchins and serves as a vital element in the medical drug called Histochrome®. EchA possesses properties that combat oxidation, reduce inflammation, and against microbial activity. However, its effects on diabetic nephropathy (DN) remain poorly understood. In the present study, seven-week-old diabetic and obese db/db mice were injected with Histochrome (0.3 ml/kg/day; EchA equivalent of 3 mg/kg/day) intraperitoneally for 12 weeks, while db/db control mice and db/m mice received an equal amount of sterile 0.9% saline. EchA improved glucose tolerance and, reduced blood urea nitrogen (BUN) and serum creatinine levels, but did not affect body weight. In addition, EchA decreased renal malondialdehyde (MDA) and, lipid hydroperoxide levels, and increased ATP production. Histologically, EchA treatment ameliorated renal fibrosis. Mechanistically, EchA suppressed oxidative stress and fibrosis by inhibiting protein kinase C-iota (PKCι)/p38 mitogen-activated protein kinase (MAPK), downregulating p53 and c-Jun phosphorylation, and attenuating NADPH oxidase 4 (NOX4) and transforming growth factor-beta 1 (TGFβ1) signaling. Moreover, EchA enhanced AMPK phosphorylation and nuclear factor erythroid-2-related factor 2 (NRF2)/heme oxygenase 1 (HO-1) signaling, improving mitochondrial function and antioxidant activity. Collectively, these findings demonstrate that EchA prevents DN by inhibiting PKCι/p38 MAPK and upregulating the AMPKα/NRF2/HO-1 signaling pathways in db/db mice and, may provide a therapeutic option for DN.

Keywords: echinochrome A, diabetic nephropathy, protein kinase C, renal fibrosis, oxidative stress

P10-13

Dual action of inorganic phosphate on podocyte filter function: A little goes a long way

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Podocytes, specialized epithelial cells forming the glomerular filtration barrier, are susceptible to malfunction due to dysregulation of intracellular Ca^{2+} signaling, resulting in actin cytoskeleton remodeling, slit diaphragm disruption, and proteinuria, a hallmark of kidney diseases. Besides the prominent role of *Trpc5/6* channels in podocyte integrity, emerging evidence suggests that Orai1-mediated store-operated Ca^{2+} entry (SOCE) also contributes to Ca^{2+}-dependent filter maintenance during podocyte injury. In the context of diabetic kidney diseases, reactive oxygen species (ROS) elevates podocyte Ca^{2+} level. Chronic kidney disease (CKD), characterized by escalated oxidative stress, is associated with hyperphosphatemia. However, the impact of excessive inorganic phosphate (Pi) on SOCE-mediated Ca^{2+} signaling and filtering function in podocytes remains poorly understood. Here we demonstrate that Pi upregulates the ER kinase PERK and the oxidoreductase ERO1α, promoting mitochondria-ER contacts, giving rise to the enhanced mitochondrial Ca^{2+} uptake, depolarization of mitochondrial membrane potential, and augmented mitochondrial ROS generation. Concurrently, Pi-induced ROS triggered ER stress and SOCE activation, leading to Akt-dependent exocytosis of Orai1 and its greater surface abundance. This dysregulation in cytosolic Ca^{2+} or ROS disrupted actin cytoskeleton and altered podocyte morphology and plasticity, causing increased albumin permeability. Notably, the inhibition of Orai1 with GSK7975A partly rescued the actin cytoskeleton disruption and synaptopodin loss. Consistently, in vivo studies using podocyte-specific *Orai1*-deletion (*Nphs2*;*Orai1*$^{fl/fl}$) mice showed reduced Pi-induced albuminuria. Short-term Pi exposure instigated the upregulation of stress markers GDF15 and FGF21 in podocytes, providing protective feedback against the fatal impact of ROS and intracellular Ca^{2+} dysregulation. However, long-term Pi exposure irreversibly deteriorated actin cytoskeleton and cell viability, likely resulting in slit diaphragm integrity loss and proteinuria, subsequently. Overall, our findings highlight the dual effects of Pi on podocytes, specifically in terms of Ca^{2+}-regulated podocyte filter function and potentially uncover therapeutic targets for proteinuria and associated renal disorders.

Keywords: albuminuria, ROS, Orai1, Ca^{2+} signaling, actin cytoskeleton

P10-14

Inhibition of c-jun N-terminal kinase prevents epithelial-mesenchymal transition and fibrosis in human podocytes

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Transforming growth factor-β (TGF-β) has been known to play critical roles in the pathogenesis of fibrotic glomerular and tubular diseases. As a non-canonical signaling of TGF-β, activation of extracellular signal-regulated kinase (ERK), followed by mammalian target of rapamycin and NADPH oxidase 4 upregulation, participates in epithelial-mesenchymal transition (EMT) and fibrosis in glomerulosclerosis *in vitro* and *in vivo* models. However, the participation of other mitogen-activated protein kinases (MAPKs) such as p38 MAPK and c-Jun N-terminal kinase (JNK) in TGF-β-induced EMT and fibrosis have remained unknown. In this study, we investigated the therapeutic potentials of p38 MAPK and JNK inhibitors in a differentiated human podocyte cell line. TGF-β upregulated mesenchymal markers, such as collagen and α-smooth muscle actin, and attenuated epithelial markers, including zona occludens-1 (ZO-1) and cadherin. In addition, TGF-β altered protein expressions related to mitochondrial Ca^{2+} uptake, fission/fusion, and autophagy in human podocytes. Pharmacologic inhibition of JNK markedly abrogated downstream TGF-β signaling and EMT, whereas p38 MAPK inhibitor did not elicit significant consequences. Interestingly, RNA interference-mediated genetic suppression of JNK3 strongly inhibited EMT and fibrosis by TGF-β compared to that of JNK1, while JNK2 knockdown did not affect it. Selective JNK3 inhibitors efficiently abolished TGF-β-induced EMT and ROS generation. Foot process effacement of podocytes by TGF-β or palmitate in electron microscopic images was remarkably protected by selective JNK3 inhibitors. Taken together, we suggest that JNK3 could be a novel and effective therapeutic target for various chronic diseases related to TGF-β-induced EMT and fibrosis.

Keywords: renal fibrosis, TGF-Beta signaling, c-Jun N-terminal kinase 3 (JNK3), glomerulosclerosis, chronic kidney disease

P10-15

The potential effect of vitamin B12 supplementation on kidney cubilin and amnionless level in rat model

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Vitamin B12 (B12) is an essential water-soluble vitamin in the process of DNA synthesis due to its function as a methyl donor in all cells of the body. This micronutrient is also crucial for maintaining low levels of homocysteine, an oxidative stress agent, to remain low in plasma and tissues. Our previous research concluded a significant relationship between vitamin B12 deficiency and impaired kidney structure and function in animal model. This study aims to evaluate the effect of VB12 supplementation on cubilin and amnionless levels in kidney tubules in experimental animal models.

This study was conducted with 39 male Wistar rats, which were divided into 3 groups: control group (C), treatment group with VB 12 per-oral 10 µg/day (PO), and treatment group with VB12 intramuscular 10 µg three times a week (IM). After 6 weeks, the animals were sacrificed, kidney tissue samples were taken and then Cubilin and Amnionless protein levels were quantified.

Kidney tissue Cubilin level was significantly higher in the control group compared to VB12 supplementation groups (C vs PO vs IM: 1.82 vs 3.9 vs 6.4 ng/dl/mg, $p<0.05$). Amnionless levels of kidney tissue were significantly lower in the treatment groups compared to the control group (C vs PO vs IM: 138.49 vs 126.61 vs 117.22 ng/dl/mg, $p<0.05$).

This study concluded a potential effect of VB12 supplementation on kidney cubilin and amnionless levels, led to the possibility of kidney physiological changes, especially in the absorption process in the renal tubules mediated by Cubilin and Amnionless.

Keywords: amnionless, cubilin, kidney, Vitamin B12

P11-1

Effects of licorice ingredients on GIRK channel activity and atrial function

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G-protein-gated inwardly rectifying K^+ (GIRK1, GIRK2, GIRK3, GIRK4 subunits) channels are expressed in various tissues and are involved in the regulation of membrane potentials. Recent studies suggest that cardiac type GIRK channels (heterotetramers of GIRK1 and GIRK4) are constitutively activated in patients with chronic atrial fibrillation. On the other hand, excess intake of licorice, a commonly used food additive and a Chinese medicine, is also known to induce atrial fibrillation. We therefore hypothesized that excess licorice intake may affect atrial function via modulation of GIRK channel activity. In this study, we investigated the effects of the main ingredient of licorice, glycyrrhizic acid (GA), and its metabolite 18β-glycyrrhetic acid (18β-GA) on GIRK channel activity by electrophysiological recordings using *Xenopus* oocytes expressing different GIRK subunits (GIRK1, GIRK2, GIRK4). We also examined their effects on atrial function by motion analyses of myocyte contraction of primary cultured neonatal rat atrial myocytes. We observed that GA inhibits the current of GIRK1-containing channels, whereas 18β-GA activates the current of all combinations of GIRK channels in a voltage-dependent manner. PIP_2 was essential for 18β-GA-evoked GIRK activation, whereas the coupling between $G_{\beta\gamma}$ and GIRK channel was not critical for the action. Mutation of Phe137, a GIRK1-specific amino acid residue in the pore helix, to Ser abolished the inhibitory effect by GA, while it potentiated the activation effect by 18β-GA. Moreover, neutralization of Glu236 located at the cytoplasmic pore of GIRK2 eliminated the 18β-GA-evoked activation, suggesting a potential role of Glu236 in the interaction with 18β-GA. Furthermore, we confirmed that 18β-GA suppresses spontaneous beating of cardiomyocytes via activation of native GIRK channels in rat atrial myocytes. Taken together, these results indicate that GA and 18β-GA have distinct actions on GIRK currents, and suggest that licorice-induced atrial arrythmias may be caused by activation of GIRK channel activity by the metabolite.

Keywords: potassium channel, licorice, atrial fibrillation

P11-2

Photoreceptor ion flux profiles and ion homeostasis in a comprehensive mathematical model

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A disturbance of photoreceptor ion homeostasis can cause photoreceptor degeneration and cell death, leading to many retinal diseases such as retinitis pigmentosa, age-related macular degeneration, and retinal detachment. Maintaining photoreceptor ion homeostasis requires large amounts of energy for the extrusion of excess ions and phototransduction cascades. However, the detailed mechanisms remain elusive. This study has constructed a mathematical model for exploring photoreceptor ion fluxes and ion homeostasis in detailed mechanisms by modeling ion concentrations (Na^+, K^+, Ca^{2+}, Cl^-) and membrane ion fluxes based on conductance-based ion channel models incorporating the classical Goldman-Hodgkin-Katz constant field equation. The proposed model consists of various ion channels, ion pumps, exchanges, and transporters, including; I_{CNG}, I_{NCKX}, I_{CaL}, I_{HCN}, I_{Kv}, I_{KCa}, I_{ClCa}, I_{NaK}, I_{PMCA}, I_{NCX}, J_{NKCC1}, J_{KCC2}, and unidentified leak channels, for maintaining ion homeostasis. Each ionic current is identified using a gene expression database. The simulation results show that I_{CNG}, I_{NCKX}, and I_{HCN} currents significantly contribute to Na^+ uptake into the cell, and their fluxes in darkness are approximately 2.1, 0.4, and 0.6 mM s^{-1}, respectively. In light, both I_{CNG} and I_{NCKX} currents are close to zero, whereas Na^+ uptake via I_{HCN} channels is raised to 2.2 mM s^{-1} for shaping membrane potential. I_{CaL} channels are the primary route responsible for Ca^{2+} uptake into photoreceptor at about 2 mM s^{-1} in darkness and 1.5 mM s^{-1} in light. A normal dark I_{NaK} current of 2.3 pA/pF and *I*PMCA current of 1.7 pA/pF play an essential role in maintaining ion homeostasis. However, these mechanisms largely utilize approximately $7x10^7$ molecules of ATP s^{-1} for active ion transports associated with the concentrations and the electrical gradients. In bright light, the photoreceptor closes I_{CNG} currents, resulting in physiological alterations of each ion flux for maintaining ion homeostasis. The ATP consumption rates through I_{NaK} and I_{PMCA} pumps also decrease by about 8.5% and 66.3% of those in darkness, respectively. In contrast, the energy requirements of the phototransduction cascade show a relatively small increase. Both decreased energy production and increased oxidative stress cause a reduced energy supply and subsequent disruption of ion

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homeostasis, leading to photoreceptor degeneration and vision loss. To date, effective therapy has not been developed for handling photoreceptor loss, but several therapeutic approaches have reported success in slowing retinal degeneration. A better understanding of the detailed photoreceptor ion flux and ion homeostasis may provide insight into the pathophysiological basis of retinal diseases and serve as a clinical practice guideline for developing a potential therapeutic approach in the near future.

Keywords: ion homeostasis, ion channels, ionic current model, mathematical simulation model, retina

P11-3

An intermediate state in the 2nd voltage sensor domain is related to the PIP_2-gating mode in two-pore channels

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Background: Two-pore channels (TPCs) are the voltage-gated cation channels essential for Ca^{2+} release from the endosomes and lysosomes. They are involved in a variety of physiological processes, diseases, and even viral infections such as SARS-CoV-2. TPCs consist of two homologous repeats, each with six transmembrane helices that correspond to a functional unit of the voltage-gated cation channel superfamily. Domain I binds PIP_2, while domain II (DII) is responsible for voltage sensing. Each TPC subtype has its own sensitivity to these two stimuli. Although many structures of TPCs in the apo- or PIP_2-bound state have been studied, it remains unclear how the TPC subtypes exhibit different sensitivity to the two stimuli.
Methods: Electrophysiological analyses of TPCs were performed in the *Xenopus laevis* oocyte expression system using the two-electrode voltage clamp technique. Two TPC subtypes, TPC3, which is primarily voltage-gated and TPC2, a PIP_2-gated and non-voltage-gated type, were analyzed to deduce whether they use a similar mechanism to sense the two stimuli. In particular, voltage clamp fluorometry (VCF) was applied to TPC3 to study the detailed dynamics of its DII-S4 helix, which is the core part for voltage sensing.
Results: VCF analysis of TPC3 revealed that DII-S4 has, in addition to up and down conformations, an intermediate state that opens in a strongly PIP_2-dependent manner. A mutation that stabilizes this intermediate state changes TPC3 from predominantly voltage-gated to strongly PIP_2-gated. In TPC2, a tricyclic antidepressant, desipramine, induces DII-S4-based voltage dependence. Mutational stabilization of the intermediate state corresponding to TPC3 impaired the voltage-gated currents in TPC2 but did not affect the PIP_2-gated currents.
Conclusions: The study revealed a unique regulatory mechanism common to TPC subtypes in which PIP_2-gating and voltage-gating modes can be switched. Like general voltage-gated ion channels, DII-S4 can adopt the resting (down) and activated (up) conformations, reflecting the voltage-gating mode. In addition, TPCs become more PIP_2-sensitive when DII-S4 is in the intermediate conformation. This suggests that DII-S4 plays a more active role than expected, which may provide new perspectives for understanding general voltage-gated ion channels.

Keywords: two-pore channel, voltage-gated ion channel, phosphoinositide, voltage-clamp fluorometry, sodium channel

P11-4

Novel binding-site of BK_{Ca} channel activator, CTIBD

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The large-conductance calcium-activated potassium channel (BK_{Ca} channel) is activated by both increasing concentration of intracellular Ca^{2+} and membrane depolarization. These channels are expressed on the urinary bladder smooth muscle and plays a critical role in relaxing smooth muscle. In our previous study, a novel BK_{Ca} channel activator, CTIBD (4-(4-(4-chlorophenyl)-3-(trifluoromethyl)isoxazol-5-yl) benzene-1,3-diol), was identified and it was confirmed that CTIBD decreases the $V_{1/2}$ and increases the G_{Max} of the BK_{Ca} channel. In this follow-up study, we investigated the binding-site of CTIBD. We identified the binding site of CTIBD and BK_{Ca} channel by using cryo-EM. The binding site is located on the turret region of the BK_{Ca} channel, which is close to the pore region. We mutated three residues located in the binding site to alanine. When those residues were changed to alanine, the BK_{Ca} channel activation effect of CTIBD decreased. The triple mutant BK_{Ca} channel showed the lowest $V_{1/2}$ shifting by 10 μM CTIBD. The triple mutant channel did not show any differences in activation and deactivation rate in the absence or presence of CTIBD. The increase in open probability by CTIBD was much lower in the triple mutant BK_{Ca} channel compared to the wild-type BK_{Ca} channel. In this study, the novel binding-site of CTIBD was identified and proven through various methods such as electrophysiology, alanine mutation study, and cryo-EM.

Keywords: ion channel, modulator, binding-site

P11-5

The distinct biophysical properties and physiological roles of two zebrafish HCN4 pacemaker channels

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The heart generates rhythmically depolarizing waves for spontaneous muscle contraction, beginning at the sinoatrial node (SAN) and propagating to the rest of the heart. Zebrafish, which are widely used as animal models for cardiovascular research, have the potential to aid in the study of inherited heart diseases, such as cardiac arrhythmia. However, it is uncertain how similar the ion channels responsible for cardiac automaticity are in zebrafish and humans. HCN4 channel is expressed in the SAN and plays a major role in spontaneous depolarization; therefore, dysfunction of the channel can cause bradycardia or sick sinus syndrome. Interestingly, zebrafish have two ortholog *HCN4* genes (*DrHCN4* and *DrHCN4L*). It is unclear whether the two HCN4 channels in zebrafish have distinct physiological functions and roles in regulating heart rate.
We first characterized the biophysical properties of the two zebrafish HCN4 channels in *Xenopus* oocytes and compared them to those of the human HCN4 channel (HsHCN4). Our findings revealed that the two zebrafish channels exhibited different gating properties; both showed faster activation kinetics and more positively shifted G-V curves than HsHCN4, and DrHCN4L showed the fastest activation kinetics and the

most positively shifted G-V curve. The intracellular region of the HCN4 channel contains a cyclic nucleotide-binding domain (CNBD), which binds to cAMP and alters the voltage dependence of the channel, playing an important role in HCN channel activation. We also created two chimeric channels (DrHCN4-4L and DrHCN4L-4) by exchanging the cytoplasmic C-terminal regions of DrHCN4 and DrHCN4L, demonstrating that the C-terminal region determines the voltage dependence.
We next carried out knockdown by antisense morpholino to determine the respective physiological functions of DrHCN4 and DrHCN4L in regulating heart rate. Knockdown of the HCN4 genes using antisense morpholino (MO) significantly reduced the heart rate; however, it returned to nearly the same level as the wild type by 72 hours post-fertilization (hpf). This indicated that the heart rate, probably by some compensation mechanisms, overcame the initial knockdown effect. Interestingly. MO knockdown caused edema around the heart during the early stages of zebrafish, suggesting their importance for early development.
In conclusion, both DrHCN4 and DrHCN4L may be required for heart rate regulation and normal cardiac development.

Keywords: HCN4 channel, zebrafish, morpholino, pacemaker channel, two-electrode voltage-clamp

P11-6

Calcium homeostasis modulator 2 (Calhm2) in mouse B lymphocyte is the slowly activating voltage-dependent channel releasing ATP

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In mouse B lymphocyte, a slowly activating voltage-dependent current (I_{VSAC}) was reported (Nam et al., 2006). Since the thermosensitivity and pH-dependence of I_{VSAC} are similar to the property of the recently cloned Calcium homeostasis modulator (CALHM) family ion channel (Jeon et al., 2021; Kwon et al., 2023), we investigated the molecular identity of I_{VSAC}. RT-PCR analysis revealed the mRNAs of Calhm2 and 6, while not Calhm1, in the primary B cells and Bal-17 cell line cells of mice. Whole-cell patch clamp study confirmed the thermosensitivity, pH-sensitivity, and the facilitation of I_{VSAC} by lowering $[Ca^{2+}]_{ext}$. The overexpressed Calhm2 in Neuro-2A cell showed the slow activating outward current with thermosensitivity, pH- and $[Ca^{2+}]_{ext}$-sensitivity. Calhm6 overexpression did not show additional membrane conductance. In Bal-17 cells, transfection with Calhm2-specific si-RNA abolished the I_{VSAC}. In mice, Calhm2 has been suggested as an ATP-releasing mechanism in astrocytes and microglia (Jun et al., 2018). In the Calhm2-overexpressed N2A cells, luciferin-luciferase assay revealed increased ATP release that was augmented by removing extracellular Ca^{2+}. Based on the above results, we suggest that Calhm2 is the molecular nature of I_{VSAC} in mouse B lymphocytes, which might provide purinergic intercellular signaling pathway.

Keywords: calcium homeostasis modulator, Calhm2, mouse, B lymphocyte, ATP release

P11-7

The analysis of lithium permeation mechanisms of prokaryotic sodium channel

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Voltage-gated sodium channels contribute to the rapid upstroke of action potentials in neuronal signal transduction, thus selective permeation of sodium ions is critical for neural activity. It has been known that lithium ions permeate sodium channels with an efficiency of about 70% of sodium ions, and drugs using lithium have been used as a treatment for manic-depressive disorder for a long time. However, the difference in the permeation mechanism between sodium and lithium ions in sodium channels has been unclear. Here, to elucidate the mechanism, we identified the lithium-ion-selectivity-enhancing mutants of NavAb, a prokaryotic sodium channel, and analyzed their functions and crystal structures.
The ion selectivity filter of NavAb consists of five amino acids (TLESW), and the center of its tetramer forms an ion pore. The small-side-chain mutations of the serine residue of the selectivity filter increased the selectivity for lithium ions. To analyze the mechanism of increased lithium selectivity, we determined the crystal structures of these mutants. In mutants with improved lithium selectivity, the entrance of the selectivity filter widened as the side chain became smaller. Remarkably, in the glycine mutant, which had the smallest side chain, water molecules, not found in the wild type, were observed at the ion entry port. Additional water molecules are located in the place generated by the glycine mutation. This result suggested that the glycine mutation allows metal cation to omit one time of the coordination exchange from the hydration water to the oxygen atom of protein residue. Because of its smaller ionic radius, lithium interacts more strongly than sodium with oxygens and hydroxyl groups of water molecules. Therefore, the hydration-water-exchange rate of lithium ions is slower than that of sodium ions. In conclusion, we hypothesized that the fewer times of coordination exchange of cation around the selectivity filter contribute to lithium selectivity. For further verification, we will analyze the molecular behavior in NavAb using kinetic calculation and molecular dynamics simulation.

Keywords: ion channel, sodium channel, ion selectivity, electrophysiology, structural biology

P11-8

Role of ANO1 in HNSCC: insights into altered expression and regulatory function in VRAC current

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The Anoctamin1 (ANO1) gene, also known as TMEM16A, encodes a calcium-activated chloride channel found in various epithelial cells. Its role has garnered attention in cancer research due to its altered expression levels. ANO1 expression is significantly amplified in several tumors, including head and neck squamous cell carcinoma (HNSCC), and knockdown of ANO1 has been shown to reduce cell migration and proliferation. However, the exact electrophysiological role of ANO1 remains unclear.

In this study, we observed high ANO1 expression in HNSCC patients, which correlated significantly with prognosis based on database analysis. To further investigate its function, we conducted whole-cell patch clamp measurements of I_{ANO1} in seven different types of cancer cell lines, including HNSCC, breast cancer (BCa), and prostate cancer (PCa). While I_{ANO1} was detected in BCa and PCa cells, it was absent in all HNSCC cell lines. Confocal imaging and immunoblot analysis of HNSCCs revealed negligible expression of ANO1 in the plasma membrane despite its high cytosolic expression. Conversely, BCa and PCa cells showed clear membrane expression, consistent with the I_{ANO1} recordings. Furthermore, in the presence of ANO1-specific inhibitors, only BCa and PCa cell lines that expressed ANO1 on their surface exhibited reduced migration and proliferation. To investigate the role of ANO1, we utilized CRISPR-cas9 to knock out ANO1 in HNSCC cell lines. As a result, we observed a decrease in volume-regulated anion channel (VRAC) current in the ANO1 knockout cell lines.
Collectively, these findings suggest that ANO1 does not function primarily as an ion channel but rather plays a regulatory role in VRAC current in HNSCC cell lines.

Keywords: ANO1, VRAC, head and neck squamous cell carcinoma

Poster

P11-9

Multi-target modulation of ion channels underlying the analgesic effects of α-mangostin in dorsal root ganglion neurons

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Background: α-Mangostin is a xanthone from the pericarps of mangosteen fruit with antinociceptive and analgesic properties. Although such effects suggest an involvement of ion channels in the nociceptive neurons, their electrophysiological investigation is lacking.
Methods: For whole-cell patch clamp study, mouse dorsal root ganglion (DRG) neurons, ND7/23 cells, and HEK293T cells overexpressed with targeted channels were used. A molecular docking (MD) simulation and *in silico* ADME analysis were conducted to get a further insight of binding sites and pharmacokinetics, respectively.
Results: Application of α-mangostin (1–3 μM) hyperpolarized the resting membrane potential of small cell DRG neuron with increased background K^+ conductance, which inhibited the action potential generation. In HEK293T cells overexpressed with TREK-1, TREK-2, or TRAAK of the two-pore domain K^+ channel (K2P) family, α-mangostin increased the outward currents at micromolar ranges. However, TRESK, another type of K2P family was not affected by α-mangostin. Notably, α-mangostin (3 μM) almost completely inhibited the capsaicin-induced TRPV1 currents, and partly suppressed the tetrodotoxin-sensitive voltage-gated Na^+ channel (Na_V) currents. MD simulation revealed that multiple oxygen atoms in α-mangostin form stable hydrogen bonds with TREKs, TRAAK, TRPV1, and Na_V channels. *In silico* ADME tests suggested that α-mangostin may satisfy the drug-likeness properties without penetrating blood-brain barrier.
Conclusion: The analgesic properties of α-Mangostin might be mediated by the multi-target modulation of ion channels; TREK/TRAAK activation, TRPV1 inhibition, and decrease of tetrodotoxin-sensitive Na_V current

Keywords: dorsal root ganglion, nociceptor, Analgesic mechanism, α-Mangostin, TRPV1, TREKs/TRAAK, voltage operated Na^+ channel

P11-10

Multiple effects of Echinochrome A on selected ion channels implicated in skin physiology

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Echinochrome A (Ech A), a naphthoquinoid pigment from sea urchins, is known to have anti-inflammatory and analgesic effects that have been suggested to be mediated by antioxidant activity and intracellular signaling modulation. In addition to the mechanisms, ion channels in keratinocytes, immune cells, and nociceptive neurons might be the target for the pharmacological effects. Here, using the patch clamp technique, we investigated the effects of Ech A on the Ca^{2+}-permeable TRPV3 and Orai1 channels, and the two-pore domain K^+ (K2P) channels (TREK/TRAAK, TASK-1, and TRESK) overexpressed in HEK 293 cells. Ech A inhibited both TRPV3 and Orai1 current with IC_{50} of 2.1 and 2.4 μM respectively. Ech A alone did not change the amplitude of TREK-2 current (I_{TREK2}), but pretreatments with Ech A markedly facilitated I_{TREK2} activation by 2-APB, arachidonic acid (AA), and acidic extracellular pH (pH_e). Similar facilitation effects of Ech A on TREK-1 and TRAAK were observed when stimulated with 2-APB and AA, respectively. On the contrary, Ech A did not affect TRESK and TASK-1 currents. Interestingly, the I_{TREK2} maximally activated by the combined application of 2-APB and Ech A was not inhibited by norfluoxetine but was still completely inhibited by ruthenium red. The selective loss of sensitivity to norfluoxetine suggested altered molecular conformation of TREK-2 by Ech A. We conclude that the Ech A-induced inhibition of Ca^{2+}-permeable cation channels and

facilitation of TREK/TRAAK K2P channels might underlie the analgesic and anti-inflammatory effects of Ech A.

Keywords: Echinochrome A, skin, ion channel, TREK/TRAAK, TRPV3, Orai1

P11-11

Mitochondrial localization of NS5806-responsive K^+ channels in HeLa cells

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Background: Mitochondria are at the center of cell physiology, energetics, and various metabolic diseases, such as neurodegenerative disease, cancer, and diabetes. The functions of the mitochondria include oxidative phosphorylation, reactive oxygen species (ROS) generation, induction of apoptosis, and mitophagy, and such functions depend on the mitochondrial membrane potential (MMP). However, only a few potassium (K^+) channels have been identified in the mitochondrial membrane (e.g., K_{ATP}, $K_v1.3$, and $K_v7.4$), despite the diversity of the K^+ channel family. In this study, NS5806-responsive K^+ channel was reported, which was found functional in HeLa cells' mitochondria.
Methods: HeLa cells were fractionated via differential or gradient centrifugation and screened for K^+ channels localized in the mitochondria using Western blot. Immunofluorescence (IF) was also examined for fixed HeLa cells. Using confocal scanning laser microscopy and flow cytometry, MMP regulation was detected by the K^+ channel modulator, NS5806, in intact HeLa cells. Using JC-1 and MitoTracker Green dyes, MMP modulation and mitochondrial matrix swelling were examined by NS5806 in the isolated HeLa mitochondria. Furthermore, the changes in oxygen consumption rate (OCR) and ATP synthesis were examined when NS5806 was treated.
Results: The NS5806-responsive K^+ channel protein was enriched in mitochondrial fraction among other K^+ channels. IF results supported the localization of K^+ channels in mitochondria. NS5806 treatment in intact HeLa cells resulted in a dose-dependent decrease of MMP within several minutes. MMP dissipation by NS5806 in the isolated mitochondria demonstrated that the effect of NS5806 was independent of the expression of NS5806-responsive K^+ channels in the plasma membrane. We could also observe the swelling of the isolated mitochondria in a dose-dependent manner, which confirms mitochondrial K^+ influx accompanied by osmotic H_2O transport. In the OCR measurement, an uncoupler-like activity of NS5806 was observed, which was evidenced by sustained oxygen consumption when ATP synthase was inhibited by oligomycin. Moreover, this activity showed a dose-dependent and highly stabilized increase of OCR. NS5806 treatment also led to a decrease in ATP synthesis with minimal cytotoxic effects.
Conclusion: A NS5806-responsive K^+ channel was discovered in the mitochondrial membrane of HeLa cells. NS5806 effectively modulated MMP, leading to downstream effects on cellular metabolism. NS5806 showed an uncoupler-like activity with high stability, indicating its therapeutic potential for ROS- or metabolism-related damages.

Keywords: K^+ channels, mitochondrial ion channels, mitochondrial membrane potential, reactive oxygen species, metabolism

P11-12

Tentonin 3/TMEM150C comprises a pore-forming subunit of a slowly-inactivating mechanosensitive channel

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Tentonin 3/TMEM150C (TTN3) is a mechanosensitive (MS) ion channel with slowly-adapting (SA) kinetics associated with several physiological relationships regarding proprioceptive sensation, baroreceptor sensitivity, and insulin secretion. Compared to other MS channels, TTN3 displays unique biophysical properties, including SA-type mechanically activated currents, and specific inhibition from a mutant conotoxin, noxious mechanosensation blocker 1 (NMB-1). Even with these distinctive characteristics, however, the structural arrangement of TTN3 as an ion channel was not yet visualized. Here, we present structural models of TTN3 as a pore-forming subunit in a tetrameric composition. Utilizing deep learning-based protein structure prediction algorithms, AlphaFold2, and molecular dynamics, the tetrameric configuration of TTN3 reveals a pore region. Amino acid residues W98, I101, V131 and F139 are key sites which establish the TTN3 pore formation. Also, mutation studies targeting the N- and C-termini of TTN3 contributes its mechanosensitivity, possibly participating on channel gating. With these structural and electrophysiological data, TTN3 is a pore-forming subunit of a slowly adapting mechanosensitive channel.

Keywords: physiology, mechanosensitive ion channel, mechanosensation

P11-13

Trp^{434} and Trp^{435} residues are crucial for sensing Calcium ions and PI(4,5)P2 molecules in TRPC5 channel

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Transient receptor potential canonical (TRPC) channels are non-selective, calcium-permeable cation channels. Among the seven subunits, TRPC4 and TRPC5 channels are well known to be potentiated by the direct binding of calcium ions and phosphatidylinositol 4,5-bisphosphate (PIP2). Both calcium and PIP2 are implicated not only in the activation of the channel but also in desensitization processes. While the binding site for calcium ions has been revealed, the PIP2 binding site remains controversial. In previous research, we identified two tryptophan residues in the S3 helix of the TRPC4 channel as potential candidates for delivering calcium sensing from the S2-S3 linker to channel gating. In this study, we suggest that the WW site in the TRPC5 channel plays a similar role in delivering calcium sensing and may also act as a potential PIP2 binding site.
To investigate the role of WW site, we created two single mutants (W434A, W435A) and a double mutant (WW/AA) from the human TRPC5 channel. We expressed the wild-type and mutant TRPC5 channels in HEK293 cells for macroscopic current recording in whole-cell or inside-out mode. All mutant channels exhibited comparable current responses to the wild-type, showing typical doubly-rectifying I-V curves of the TRPC5 channel when stimulated by the non-physiological agonist, (-)-Englerin-A (EA), in whole-cell mode. However, under stimuli mimicking the physiological environment, W434A showed a gain-of-

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function phenotype, while W435A and the double mutant showed loss-of-function phenotypes. Interestingly, when the W434A channel was co-expressed with human muscarinic acetylcholine receptor type 3 (mAChR3) and stimulated by carbachol, it did not produce the desensitization that is characteristically observed in the wild-type TRPC5 channel. To investigate the direct interaction with PIP2, we delivered a water-soluble form of PIP2 (diC8-PI(4,5)P2) to the intracellular side of the patch in inside-out mode. As a result, W434A and W435A channels were not potentiated by intracellular PIP2.
The loss of desensitization in the W434A mutant channel implies that this mutant has lost the ability to sense PIP2 hydrolysis by phospholipase C (PLC) or the ability to be phosphorylated by protein kinase C (PKC). The latter possibility is ruled out because the C-termini of the channels we used were truncated, so they do not contain the phosphorylation site. Recently, PIP2-bound ion channel structures, including TRP channels, have been revealed. Many of them have PIP2 binding sites near the S3, S4, and S5 helices. Our electrophysiological data also indicate that PIP2 binds near the S3 helix in the TRPC5 channel, and the WW site is a specific candidate for sensing PIP2 and calcium.

Keywords: TRPC channel, calcium, PIP2

Poster

P11-14

Bidirectional sensitivity of CALHM1 channel to protons from both sides of plasma membrane

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Calcium homeostasis modulator 1 (CALHM1), a newly discovered voltage-dependent nonselective ion channel, has drawn attention for its role in neuronal activity and taste sensation. Its sluggish voltage-dependent activation is facilitated by lowering extracellular Ca^{2+} concentration ($[Ca^{2+}]e$). Here, we investigated the effects of extracellular and intracellular pH (pHe and pHi) on human CALHM1. When normalized to the amplitude of the CALHM1 current (ICALHM1) under whole cell patch clamp at symmetrical pH 7.4, ICALHM1 decreased at acidic pHe or pHi, whereas it sharply increased at alkaline pHe or pHi. The effects of pH were preserved in the inside-out configuration. The voltage dependence of ICALHM1 showed leftward and rightward shifts at alkaline and acidic pHe and pHi, respectively. Site-directed mutagenesis of the water-accessible charged residues of the pore and nearby domains revealed that E17, K229, E233, D257, and E259 are nonadditively responsible for facilitation at alkaline pHi. Identification of the pHe-sensing residue was not possible because mutation of putative residues impaired membrane expression, resulting in undetectable ICALHM1. Alkaline pHe-dependent facilitation appeared gradually with depolarization, suggesting that the sensitivity to pHe might be due to Hþ diffusion through the open-state CALHM1. At pHe 6.2, decreased $[Ca^{2+}]e$ could not recover the inhibited ICALHM1 but further augmented the increased ICALHM1 at pHe 8.6, suggesting that unidentified common residues might contribute to the $[Ca^{2+}]e$ and acidic pHe. This study is the first, to our knowledge, to demonstrate the remarkable pH sensitivity of CALHM1, which might contribute to the pH-dependent modulation of neuronal excitability or taste sensation.

Keywords: CALHM1, pH sensitivity, voltage-dependent activation, pH-sensing residues, proton-calcium

P11-15

Cryo-EM structure and function of a calcium-activate chloride channel best1 in a wide-open state

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Bestrophin-1 (BEST1) is a Ca^{2+}-activated chloride channel involved in various physiological processes including Ca^{2+} homeostasis, cell volume regulation, and gliotransmitter release. The structures of chicken BEST1 and human BEST1 (hBEST1) have shown that direct Ca^{2+}-binding leads to the opening of a narrow channel pore to transport Cl^-. However, these structures are not sufficient to provide structural insight to understand how BEST1 transports small monovalent Cl^- and large gliotransmitters. To understand ion transport mechanism of BEST1, we aimed to solve the molecular structure and characterize the ion permeability of hBEST1 channel. We have solved cryo-EM structures of hBEST1 in closed, intermediate, and wide-open conformations at the resolution of 2.42~2.98 Å. These structures provide three essential findings. First, hBEST1 undergoes large conformational changes of the transmembrane (TM) domain, which induces wider pore opening than previously suggested open conformation. In the wide-open structure, the whole TM helical bundles of each protomer are rotated ~32° in a rigid body movement instead of local rotation of the pore-lining helix, S2a. Second, multiple conformations visualize electron densities along the central axis of the pore, which may be permeant Cl^-. Third, a conserved Gln, Q208 in the cytoplasmic domain coordinates a presumably Cl^-. Indeed, Q208 mutations showed altered current density as well as anion selectivity, especially for large organic anions. Currently, we are examining the electrophysiological characteristics of mutant channels inspired by cryo-EM structures.

Keywords: Bestrophin, Cryo-EM, mechanism

P11-16

n-Alcohol modulation of action potential firing depends on Kv7.2/7.3 channel regulation

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Action potential (AP) is one of the critical electrophysiological properties of neurons and is produced by the interaction of several finely tuned ion channels. The opening and closing of ion channels create a delicate balance between the various ion channels to maintain homeostasis at resting potentials and to induce electrical activity during action potentials. Here, we provide evidence that the modulatory effect of *n*-alcohols on neuronal firing is dependent on the regulation of potassium channels rather than sodium channels. Using a primary cultured superior cervical ganglion (SCG) neuronal system, we found that the short-chain *n*-alcohol, ethanol, and the long-chain n-alcohol, hexanol, modulated action potential firing differently, where ethanol increased the AP firing while hexanol inhibited it. Both ethanol and hexanol commonly reduced the amplitude of action potentials and decreased the sodium currents of SCG neurons in a concentration-dependent. However, we found that Kv7 currents were differently regulated by the two alcohols; Kv7 current amplitudes were decreased by ethanol, but increased by hexanol. When comparing the effects of *n*-alcohols with various potassium and sodium channel regulators, it was confirmed that other types

of K^+ channels, except Kv7.2/7.3, were also involved in the alcohol regulation of neuronal firing in SCG neurons. Together the results uncovered the regulatory action mechanism of alcohol on neuronal excitability and provided new insights into drug development and pain treatment strategies.

Keywords: action potential, n-alcohol, superior cervical ganglion (SCG), Kv7.2/7.3 channels

P11-17

Molecular basis of L-type CaV1.2 channel regulation by phosphatidylinositol 4,5-bisphosphate

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Intracellular Ca^{2+} is important for regulating several cellular activities, including muscle contraction, neurotransmitter release, hormonal secretion and neuronal excitation. Voltage-gated Ca^{2+} channels (VGCC) control the entry of Ca^{2+} and various subtypes are reported. VGCCs exist in several regions, and among them, L-type Ca^{2+} channels ($Ca_V1.2$) are especially in heart and neurons. There have been several researches about phosphatidylinositol 4,5-bisphosphate (PIP_2) regulation on L-type $Ca_V1.2$ channel, which revealed that PIP_2 stabilizes the channel gating and possible binding of PIP_2 in I-II loop of É'1 pore-forming subunit. We have been further conducted experiments to quantitate the effect of PIP_2. We first constructed PIP_2-insenstive form of $Ca_V1.2$ by substituting four basic motif on C-terminus of I-II loop to neutral alanine amino acid possibly to disrupt the interaction between PIP_2 and I-II loop. In tsA-201 cells transfected with the mutant É'1C construct with Ca_V β and Ca_V É'2δ1, Ba^{2+} current was measured by whole-cell patch clamp. We found that there was no more inhibition of current by the activation of voltage-sensing phosphatase from Danio Rerio (Dr-VSP) which depletes PIP_2. Voltage-dependent activation and inactivation were measured for gating properties. It showed reduced current density of PIP_2-insensitive $Ca_V1.2$ and slight rightshit of voltage-dependent inactivation. Recently, it has been reported that PIP_2 regulates N-type Ca^{2+} channel ($Ca_V2.2$) through interaction with domain II voltage-sensor ($S4_{II}$) of É'1B subunit. In case of $Ca_V1.2$, mutation of the domain II voltage-sensor ($S4_{II}$) did not affect the current amplitude and PIP_2 regulation. Together we conclude that L-type $Ca_V1.2$ channel interacts with PIP_2 only through the I-II loop C-terminus and the regulatory mechanism of PIP_2 is different depending on the Ca_V channel subfamily.

Keywords: L-type Ca^{2+} Channels, PIP2, PIP2 binding site, Dr-VSP, PIP2 sensitivity

P11-18

Molecular mechanisms on proton-activated chloride (PAC) channel modulated by membrane $PI(4,5)P_2$

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Extracellular acidification causes the activation of proton-activated chloride (PAC) channel, thereby playing important role in involving in acid-induced cell death and regulating endocytic pathway. In addition to proton, other factors that regulate the opening of PAC channel are largely unknown. Here, we identified that the change of phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) levels through *Danio rerio* voltage-sensitive phosphatase (Dr-VSP) regulates PAC1 and PAC2 channel activation. This regulation also appears via the activation of muscarinic receptor, a Gαq protein-coupled receptor, with signaling pathway that hydrolyzes $PI(4,5)P_2$ in both transiently and endogenously expression system. We further demonstrate that PAC channel shows $PI(4,5)P_2$-dependent regulation, not PI4P. The single point mutation of basic amino acids located at the interface between transmembrane domain (TMD) 2 and C-terminus adjacent to inner plasma membrane significantly attenuates PAC channel activity. Docking simulation based on Cyro-EM structure reveals that the putative $PI(4,5)P_2$-interacting sites of PAC channel become close to the cell membrane owing to conformational change in activated state, increasing the possibility to bind to $PI(4,5)P_2$. Mutants on $PI(4,5)P_2$-binding sites reduced acid-induced cell death due to suppressed PAC channel activity, indicating that regulation of PAC channel by $PI(4,5)P_2$ affect cell death induced by acid treatment. Our study proposes that membrane $PI(4,5)P_2$ is important key factor understanding PAC channel gating.

Keywords: proton-activated chloride channel, PAC channel, TMEM206, PI(4,5)P2, phospholipid

P11-19

TRPC6 deficiency induces adipocyte dysfunction and phenotypic traits resembling obesity

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The perturbation of calcium signaling has been implicated in metabolic disorders such as obesity and diabetes. Here, we investigated the impact of TRPC6 deficiency on adipose tissue lipid metabolism and its association with obesity. TRPC6 knockout (TRPC6 KO) mice exhibited increased body weight, reduced respiratory quotient, oxygen consumption, locomotor activity, and heat production compared to wild-type mice. TRPC6 depletion disrupted lipid metabolism within adipose tissues, leading to obesity. Remarkably, TRPC6 KO mice showed reduced food intake despite their obese phenotype, suggesting metabolic disorder independent of changes in feeding behavior. Associated with excessive adiposity, TRPC6 KO mice also exhibited glucose intolerance and insulin resistance. TRPC6 deficiency affected the major lipid storage tissues including adipose tissues, liver, and skeletal muscle, with tissue- and age-dependent obesity development. White adipose tissue (WAT) and brown adipose tissue (BAT) were primarily affected, with reduced integrity and hypertrophic adipocytes. Adipogenic markers were significantly reduced in TRPC6 KO adipose tissues. *In vitro* differentiation of TRPC6 KO mouse-derived pre-adipocytes showed diminished adipogenesis capacity, enlarged lipid droplets, impaired mitochondrial function, and increased reactive oxygen species production compared to wild-type counterparts. RNA-sequencing revealed perturbations in cAMP signaling in TRPC6 KO mice, a crucial pathway for adipogenesis. TRPC6 activity may play a role in regulating cAMP signaling and the activity of β-adrenergic receptors and adenylyl cyclases, contributing normal adipocyte adipogenesis. This study provides evidence linking adipocyte function, adipogenesis, and Ca^{2+} signaling through the TRPC6 channel.

Keywords: adipogenesis, lipid metabolism, cAMP signaling, calcium signaling

P11-20

Inhibition of resurgent Na^+ currents by rufinamide

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Na^+ channels are essential for the generation of action potentials in most neurons. After opening by membrane depolarization, Na^+ channels enter a series of inactivated states (e.g. the fast, intermediate, and slow inactivated states; or If, Ii and Is). If the inactivated Na^+ channel recovers via the open state upon subsequent membrane hyperpolarization, there will be "resurgent" Na^+ currents during the hyperpolarization phase, setting an imperative base for densely repetitive or burst discharges. We found that Ii is the major inactivated state responsible for the generation of resurgent currents. Rufinamide, in therapeutic concentrations, could selectively bind to Ii to slow the recovery process and dose-dependently inhibit resurgent currents. Phenytoin and lacosamide, the other two Na^+ channel-inhibiting antiseizure medications selectively binding to If and Is, respectively, fail to show a similar inhibitory effect on resurgent Na^+ currents. The molecular action of slowing of recovery from inactivation by binding to Ii also explains the highly correlative inhibitory effect of rufinamide on both transient and resurgent Na^+ currents. The modest but correlative inhibition of both currents may make a novel synergistic effect and thus strong-enough suppression of repetitive and especially burst discharges. Rufinamide may thus have a unique therapeutic spectrum for disorders with excessive neural excitabilities.

Keywords: gating, intermediate inactivated state, antiseizure medications, phenytoin, lacosamide

P11-21

TRPML3 activation by PI3P is regulated by the scramblase ATG9A in autophagy

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TRPML3 functions downstream of PI3P in the phagophore to provide Ca^{2+} for autophagosome formation. TRPML3 binds to PI3P in both the cytosolic and luminal leaflets, which induces Ca^{2+} release via TRPML3 upon autophagy induction to increase autophagy. Since ATG9A serves as a scramblase to transport PI3P from the cytosolic to luminal leaflet of the phagophore, we hypothesized that TRPML3 may interact with ATG9A to be activated by PI3P of the both leaflets in autophagy. We found that TRPML3 interacts with ATG9A at its N-terminus, and the interaction is required for not only TRPML3 activation but also autophagosome formation upon autophagy induction. Lipid delivery experiments revealed that the TRPML3-ATG9A interaction allows PI3P to be supplied to the luminal leaflet for TRPML3 activation in autophagy. Using ATG9A mutants lacking scramblase function, we confirmed that ATG9A-mediated PI3P translocation is responsible for TRPML3 activation by PI3P in autophagy. These findings suggest that TRPML3 is activated by PI3P translocation through interaction with ATG9A to increase autophagy.

Keywords: Autophagy, TRPML3, ATG9A, scramblase, PI3P

P11-22

Development of parameter optimization method to predict ionic current composition of cardiomyocyte with selective channel-blockade

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Mathematical models of cardiomyocyte are expected to be useful in predicting drug action. Therefore, we developed a computer algorithm that automatically optimizes those parameters by decreasing the Mean Squared Error (MSE) between the target (experimental) AP waveform and the model output (PO method). In this method an arbitrary AP waveform was prepared by randomizing several model parameters. And we confirmed that the PO run corrected the randomized AP shape to recover the standard AP form by modifying the scaling factors of the model parameters. In the present study, we applied this method to predict drug action. Firstly, we tested the prediction accuracy of the PO method on selective blockade of a certain channel species using model waveform (model vs model test, MM test). In this test we decreased the conductance of one channel of the target AP waveform to a certain extent and tested whether PO Method can decisively determine which channel and to what extent the conductance was decreased. However, in MM test using a single waveform, the IK_r parameter yielded fairly accurate results, whereas the other channel parameters often yielded fluctuated results from trial to trial, and that selective blocks could not be reproduced correctly. This result may suggest that the accuracy of the PO method using a single waveform is clearly limited due to the lack of optimization constraints. Therefore, we assumed that the conductance of channels other than the target channel was intact before and after the selective blockade, and applied the PO method simultaneously to two waveforms before and after the block. As a result of the two-waveform PO run, the selectively-blocked channel and the extent of the blockade were successfully determined not only for the IK_r parameter, but also for other optimized channel parameters. For the application of this method to the experimental waveforms, the assumption that the conductance of channels other than the target channel are intact before and after the selective blockade as to be satisfied. Therefore, we used continuous records of experiments in which one ion channel species was selectively blocked in a certain human iPS cell-derived cardiomyocyte. In the present study, we demonstrate the results of applying the PO method to two waveforms, one before and the other after E-4031(a selective IK_r blocker) application, simultaneously.

Keywords: mathematical models of cardiomyocyte, parameter optimization, optimization constraints, selectively-blocked channel, two-waveform PO method

P11-23

Membrane potential modulates ERK activity

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Although there is not much known about physiological function of membrane potential in non-excitable cells, membrane potential has

been linked to cell survival and proliferation. However, there is no evidence that showed that membrane potential is directly connected to proliferative signal such as ERK.
Here we show that ERK activity is regulated by the membrane potential at the single cell level in the absence of growth factors. The ERK activity was monitored by the FRET imaging using an ERK biosensor called EKAREV while controlling the plasma membrane potentials by either extracellular potassium concentration or patch clamping. The ERK activity was upregulated when membrane was depolarized by either method. The upstream MAPK cascade was also upregulated by membrane depolarization. The voltage-dependent ERK activation was diminished by depletion of phosphatidylserine, but not by removal of extracellular calcium. This suggests that phosphatidylserine dynamics is involved in the voltage-dependent ERK activation while the entry of extracellular calcium is not. This study suggests that membrane potential has a hitherto less explored function other than the well-established role in generating action potentials in excitable cells.

Keywords: membrane potential, ERK, imaging

P11-24

SREBP-1c deficiency ameliorates hepatic steatosis and liver injury in non-alcoholic steatohepatitis through lipocalin-2 mediated hepatic iron overload

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Sterol regulatory element-binding protein (SREBP) are a family of transcription factors involved in the biogenesis of cholesterol, fatty acids and triglycerides. However, the molecular mechanism and function by which SREBP-1c regulates hepatic stellate cells (HSCs) activation in non-alcoholic steatohepatitis (NASH) animal models and patients have not been fully elucidated. In this study, we explored the role of SREBP-1c on NASH and LCN2 gene expression regulation. Wild-type and SREBP-1c knockout (KO) mice fed with a high-fat/high-sucrose diet, carbon tetrachloride (CCl_4)-treated, and with lipocalin-2 (LCN2) overexpression. The role of LCN2 in NASH progression was assessed using bone marrow-derived macrophages, primary hepatocytes, and human HSCs. LCN2 expression was examined in samples from normal patients and those with NASH. LCN2 gene expression and secretion increased in CCl_4-induced liver fibrosis mice models, and SREBP-1c regulated LCN2 gene transcription. Moreover, treatment with holo-LCN2 stimulated intracellular iron accumulation and fibrosis gene expression in LX-2 human HSCs, but this effect was not observed in SREBP-1c KO HSCs, indicating that SREBP-1c-induced LCN2 expression and secretion stimulate HSCs activation through iron accumulation. Further, LCN2 expression was strongly correlated with inflammation and fibrosis in patients with NASH. Our findings indicate that SREBP-1c regulates Lcn2 gene expression, contributing to diet-induced NASH. Reduced *Lcn2* expression in SREBP-1c KO mice protects against NASH development. Therefore, the activation of *Lcn2* by SREBP-1c establishes new connection between iron and lipid metabolism, affecting inflammation. These findings may lead to new therapeutic strategies for NASH.

Keywords: SREBP-1c, non-alcoholic steatohepatitis, hepatic stellate cells, lipocalin-2

P11-25

Dysregulation of the NOTCH1 signaling pathway by Caveolin-2 in vascular endothelial cells

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Aging is a major risk factor for common neurodegenerative diseases. Although multiple molecular, cellular, structural, and functional changes occur in the brain during aging, the involvement of caveolin-2 (Cav-2) in brain aging remains unknown. We previously investigated Cav-2 expression in the brains of aged mice and its effects on endothelial cells. HUVECs showed decreased THP-1 adhesion and infiltration when treated with Cav-2 siRNA compared to control siRNA. In contrast, Cav-2 overexpression increased THP-1 adhesion and infiltration in HUVECs. Increased expression of Cav-2 and Iba-1 was observed in the brains of old mice. Moreover, there were fewer Iba1-positive cells in the brains of aged Cav-2 knockout (KO) mice than in the brains of wild-type aged mice. The levels of several chemokines were higher in the brains of aged wild-type mice than in young wild-type mice. Furthermore, chemokine levels were significantly lower in the brains of young mice, as well as aged Cav-2 KO mice, compared to wild-type aged mice. Additionally, we found that overexpression of Cav-2 in HUVECs inhibits the NOTCH1 signaling pathway involved in the cell proliferation and differentiation of endothelial cells. Collectively, our results suggest that Cav-2 expression increases in the endothelial cells of the aged brain and induces vascular dysfunction by modulating the NOTCH1 signaling pathway.

Keywords: aging, endothelial cells, caveolin-2

P11-26

Keratinocytes differentiation is modulated by carvacrol through the TRPA1-mediated Ca^{2+} signaling

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Background: Carvacrol is a main component of oregano oil and is known to possess anticancer, antimicrobial, and antioxidant effects. However, the cellular effect of carvacrol remains obscure in keratinocytes. It has been known that calcium plays an important role on the proliferation and differentiation in keratinocytes. Therefore, we hypothesized that carvacrol could induce keratinocyte differentiation through calcium signaling.
Methods: HaCaT keratinocyte was exposed to carvacrol for 24 h. Keratinocyte differentiation marker and apoptotic marker mRNA levels were analyzed by real-time PCR. Calcium signaling, pH-based transporter activity, and western blotting were examined.
Results: The mRNA expression levels of keratinocyte differentiation markers (Loricrin and Filaggrin) and apoptotic marker Bax were upregulated by carvacrol treatment for 24 h. Carvacrol-mediated Bax expression was downregulated by ERK inhibition. Carvacrol stimulation enhanced NBC activity and cell migration in HaCaT keratinocytes. Carvacrol is known to TRPA1 agonist. Thus, carvacrol stimulation revealed a calcium peak. Co-stimulation with NAC, BAPTA-AM, and 2-APB did not affect carvacrol-mediated calcium concentration. Moreover, carvacrol-mediated calcium concentration was reduced in the calcium-free condition with EGTA in HaCaT keratinocytes.

Conclusion: Carvacrol stimulation mediated keratinocyte differentiation and migration through TRPA1-mediated calcium increase in HaCaT keratinocytes.

Keywords: carvacrol, keratinocyte differentiation, calcium, migration

P11-27

The effect of pulsed electromagnetic field stimulation of live cells on intracellular Ca^{2+} dynamics changes notably involving ion channels

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Pulsed electromagnetic field (PEMF) therapy is a non-invasive treatment with promising therapeutic efficacy for bone- and cartilage-related pathologies. Certain applications of PEMF, such as osteoarthritis pain attenuation, have been widely researched. Nonetheless, the underlying mechanism of PEMF's effect when used as treatment remains elusive. In this study, the effect of PEMF stimulation on living cells in terms of intracellular calcium ($[Ca^{2+}]_i$) ions, and gene- and protein expression were measured using KG-1 cells (a human myelomonocytic cell line) and HUVECs (human umbilical vein endothelial cells). In both the KG-1 and HUVECs, PEMF stimulation resulted in enhanced Ca^{2+} influx via purinergic receptor channels, Ca^{2+} release from intracellular store, and store-operated Ca^{2+} entry into the cells. Our study revealed that PEMF controls intracellular Ca^{2+} regulation through specific ion channels, depending on the cell type. We established that it is necessary to precisely control the intensity and duration of PEMF, depending on the condition being treated and the type of tissue.

Keywords: ion channels, intracellular calcium, KG-1 cell, HUVECs, PEMF

P11-28

Crosstalk between synoviocytes and matured osteoclasts though the calcium and cytokines

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Background: Rheumatoid arthritis is an autoimmune disease caused by immune cytokine response. Fibroblast-like synoviocytes (FLS), which are activated by cytokines, is critical cells that exacerbate rheumatoid arthritis. Increased imflammatory cytokines enhance the activity of osteoclasts and osteoclast differentiation gene in joints. In this study, we verified the crosstalk between FLS and osteoclasts.
Methods: FLS, Raw 264.7, and primary isolated bone marrow-derived macrophages (BMM) cells were used. Trap staining and pit assay were performed to determine osteoclast activity. NBC activity was measured to determine the migration of matured osteoclast. NBC activity measured the changes pH_i with BCECF-AM.
Results: TNF-α and 3 mM Ca^{2+} treatment enhanced M-CSF gene expression, NBC activity, and migration in FLS. In addition, inflamed FLS-mediated factors enhanced the osteoclast differentiation factor NFATc1 staining and protein expression. Additionally, trap-positive, and bone resorption activity were increased in activated osteoclasts by FLS-mediated factors. Bone absorptive role of osteoclasts induced the releasing Ca^{2+} from dentin disc and these releasing components from osteoclast media induced enhanced migration in FLS. Treatment of BAPTA for Ca^{2+} chelation inhibited the osteoclast releasing factor-exposed FLS migration.
Conclusion: Our results address that inflamed FLS-mediated factors and increased Ca^{2+} lead to osteoclast differentiation and subsequently differentiated osteoclasts induce bone resorption ability. Releasing factor such as Ca^{2+} from osteoclast also affects FLS migration.

Keywords: rheumatoid arthritis, FLS, osteoclast, bone resorption, NBC

P11-29

Trpc mediated calcium influx in myoblasts is regulated by PDLIM5 scaffold protein

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Myogenesis is the formation of new Skeletal muscle fibers through the fusion of precursor myoblasts into multinucleated myotubes.
The store operated calcium (Ca^{2+}) entry (SOCE) in myoblasts is essential for myogenesis. Transient Receptor Pore C (TRPC) channels are a major calcium entry pathway in myoblasts. How the activity of TRPC channels is regulated in myoblasts remain elusive. Some TRPC isoforms (TRPC4 and TRPC5) own PDZ-binding domain at their C-termini suggesting a potential for protein-protein interaction particularly with scaffold proteins. ENH1 (PDLIM5) is a PDZ-LIM scaffold protein that organizes signaling events in skeletal and cardiac muscles as well as in neurons. PDLIM5 anchors various signaling and transcriptional regulators, including Protein Kinase C (PKC) isozymes at various subcellular locations. We investigated whether ENH1 could regulate the activity of TRPC channels in myoblasts.
TRPC channels activity was monitored by Ca^{2+} imaging in C2C12 cells, a model of mouse myoblasts. Thapsigargin-induced SOCE was repressed by TRPC known inhibitors, 200 μM Gd^{3+} and 50 μM 2-APB, confirming that TRPC channels is the major Ca^{2+} entry pathway in C2C12 myoblast. Then, we tested whether ENH1 and its skeletal muscle specific short splice variant, ENH4, also regulates the TRPC-mediated SOCE. Overexpression of ENH1 resulted in an almost doubled SOCE response. This increased SOCE amplitudes was also significantly repressed by TRPC inhibitors Gd^{3+} and 2-APB. Interestingly, the overexpression of ENH4 splice variant strongly prevented the SOCE response. The ENH1 overexpression-induced SOCE response was prevented by the PKC inhibitor, Gö6983 (100 nM) in myoblasts. Immunoprecipitation experiment showed a physical interaction between ENH1 and TRPC1 isoform in C2C12 myoblasts. Taken all together, these results suggest that ENH1 scaffold PKC to TRPC channels to regulate their activity and the SOCE response in myoblast.

Keywords: skeletal muscle, TRPC, PDLIM5, SOCE

Poster

P11-30

Oxidative stress-induced calcium dyshomeostasis by epigenetic alterations of TRPC1 gene contribute to the progression of Huntington's disease

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Huntington's disease (HD) is a progressive neurodegenerative disorder characterized by an expansion of polyglutamine residues in the huntingtin protein (Htt). Although dysfunctional calcium (Ca^{2+}) signaling is implicated in HD pathogenesis, the transcriptional defects affecting proteins involved in intracellular calcium homeostasis remain poorly understood. Here, we investigated the role of redox-sensitive canonical transient receptor potential (TRPC) channels in HD using STHdhQ111/Q111 (Q111) mutant Htt knock-in cells, in comparison to wild-type STHdhQ7/Q7 (Q7) cells.

Treatment with diamide, an inducer of intracellular glutathionylation and oxidative stress, resulted in the death of Q111 cells. The half-maximal inhibitory concentration (IC_{50}) of diamide was 345.6 μM for Q111 cells, while it was 524.8 μM for Q7 cells, demonstrating greater cytotoxicity in Q111 cells. Notably, Q111 cells treated with 300 μM diamide exhibited 40% cell viability after 6 hr, which was restored by the administration of N-acetylcysteine (NAC) to reduce oxidative toxicity.

Through RNA sequencing (RNA-Seq) analysis, we identified differentially expressed genes (DEGs) in Q111 cells, revealing their susceptibility to Ca^{2+}-mediated toxicity induced by oxidative stress. In our RNA-seq data, Q111 exhibited a slight increase in TRPC5 expression, a key regulator of extracellular calcium influx, compared to Q7, while concurrently displaying a notable decrease in the expression of TRPC1, which modulates inward current of TRPC5. These findings were consistent with publicly available data from HD patient striatum tissues.

To investigate spatiotemporal Ca^{2+} dynamics under oxidative stress, we examined diamide-induced Ca^{2+} mobilization, encompassing cellular influx to endoplasmic reticulum (ER) refilling, in Q111 HD cells. Treatment with Englerin A (EA), a selective agonist of TRPC5, significantly increased intracellular calcium concentration in Q111 cells (145.20 ± 10.95 nM) compared to Q7 cells (80.85 ± 9.28 nM). Remarkably, this increase in calcium was reduced to 41.34 ± 3.791 nM by a 10 nM TRPC5 antagonist, highlighting the role of TRPC5 in modulating calcium levels. Furthermore, pharmacological inhibition of TRPC5 attenuated diamide-induced apoptosis by restoring elevated Ca^{2+} levels and signaling.

Our results indicate that the aberrant expression of TRPC1/C5 genes, triggered by the inherited mutant Htt, accelerates and exacerbates HD progression. These findings underscore the critical role of TRPC1/C5 channels in HD pathogenesis and suggest that timely clinical therapeutic intervention targeting these channels may ameliorate HD pathology. Our study provides valuable insights into the intricate interplay between dysregulated Ca^{2+} homeostasis, oxidative stress, and TRPC channels, paving the way for the development of novel therapeutic strategies for HD.

Keywords: Huntington's disease, TRPC1, calcium signaling, epigenetic dysregulation, apoptosis

P11-31

Etoposide-induced gene 2.4 as a regulator of STIM1 for stored-operated calcium entry regulation

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Store-operated calcium entry (SOCE) is vital for maintaining cellular Ca^{2+} homeostasis in non-excitable cells. This process is evoked by the ER Ca^{2+} depletion leading to the translocation of ER Ca^{2+} sensor STIM1, which activates the Orai1 pore-forming channel, also known as the Ca^{2+} release-activated Ca^{2+} (CRAC) channel. Ei24 (etoposide-induced gene 2.4 kb), a resident protein in the ER membrane, has been identified as a regulator of Ca^{2+} handling between the ER and mitochondria at the mitochondria-associated membrane (MAM) by interacting with ER membrane Ca^{2+} channels. However, the impact of Ei24 on Ca^{2+} influx through plasma membrane Ca^{2+} channels, such as the CRAC channel, remains unclear. In this study, we examined the role of Ei24 in regulating SOCE by Ei24 by interacting with STIM1. Patch-clamp experiments revealed that Ei24 overexpression impaired the CRAC channel currents. Additionally, Ei24 knockout using CRISPR/Cas9 exhibited a significant augmentation in the SOCE response. Co-immunoprecipitation experiments revealed a physical interaction between Ei24 and STIM1 at the CRAC activation domain (CAD). Moreover, FRAP (fluorescence recovery after photobleaching) assays indicated that Ei24 overexpression restricts the mobilization kinetics of STIM1. These findings highlight the crucial role of Ei24 in directing the Ca^{2+} sensor STIM1 for optimal regulation of SOCE.

Keywords: CRAC channel, Orai1, CAD domain, FRAP assay, ER-PM contact site

P11-32

WNKs regulates autophagy via lysosomal TRPML1 channel

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Autophagy is a crucial cellular degradation pathway that maintains cellular physiology and facilitates adaptation to metabolic stress. The TRPML1 lysosomal Ca^{2+} release channel plays a pivotal role in initiating autophagy. WNK kinases are known regulator of multiple ion channel/transporter homeostasis, particularly Na^{+} and K^{+}. WNK1 have been implicated in autophagy inhibition through the suppression of the class III phosphoinositide-3-kinase complex. However, the precise contribution of WNK signaling to TRPML1 regulation in context of autophagy remains unclear. In this study, we discovered that WNK kinases suppressed TRPML1, resulting in autophagy inhibition. Through experiments conducted on HEK293 cells or HeLa cells expressing GCaMP3-labelled TRPML1, we observed that the overexpression of WNK1 or 4 led to the suppression of TRPML1-mediated peri-lysosomal Ca^{2+} release, indicating that multiple WNK kinases function as TRPML1 regulators. Notably,

the suppression of Ca^{2+} release and subsequent nuclear translocation of TFEB by WNK1 were rescued by forced expression of a catalytically inactive mutant of WNK1 (kinase-dead mutant, K233M), emphasizing the crucial role of catalytic activity of WNK1 in TRPML1 activation. Furthermore, insulin, as an endogenous WNK1 activator, suppressed TRPML1-mediated Ca^{2+} release. This inhibition was effectively reversed by pretreatment with WNK463, an WNK inhibitor or diC-16-PI(3,5)P_2. These results provide further support for the notion that WNK1 inhibits TRPML1 activity via suppression of class III phosphoinositide-3-kinase. Overall, our findings offer novel insights into the role of WNK kinase signaling in autophagy regulation, specifically targeting TRPML1-mediated Ca^{2+} regulation and lysosomal biogenesis.

Keywords: TFEB, lysosomal biogenesis, lysosomal Ca^{2+}

P11-33

Palmitoylation of M_1 muscarinic receptor and its functional roles

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Palmitoylation, one of the representative post-translational modifications (PTMs), is a reversible process regulated by palmitoylation or depalmitoylation enzymes. Previous research revealed that palmitoylation is important for protein quality controls, trafficking, and localization to microdomains. In this study, we found that M_1 muscarinic receptor (M_1R) possesses a cytosolic cysteine residue that can be palmitoylated. By tagging the cytosolic domains of M_1R with mCherry, we confirmed that the C-terminal domain was targeted to the plasma membrane while the other domains were located in the cytosolic region. This PM localization of C-terminus was failed when the palmitoylation blocking reagent 2-bromopalmitate (2-BP) was applied. Since there are three cysteine residues in the C-terminus, we mutated each cysteine residue to alanine and examined the cellular distribution of the domain. mCh-C421A-C-terminus and mCh-C460A-C-terminus constructs were still trafficked to the plasma membrane, but mCh-C435A-terminus was found in the cytosol, suggesting that C435 is the palmitoylation site. To investigate the functional effect of palmitoylation on M_1R, each of the three cysteine residues in C-terminus was mutated to alanine, respectively. WT, C421A, C435A and C460A M_1Rs were all expressed through the plasma membrane without significant difference in tsA201 cells. However, cytosolic translocation of the PIP_2 probe $PH_{PLC\delta1}$-GFP by muscarinic agonist oxotremorine was significantly decreased in cells transfected with C435A construct. Consistently, in electrophysiological experiments, the suppression of KCNQ2/3 channel by oxotremorine was reduced approximately by 50% in cells expressing C435A. Together, our results suggest that palmitoylation of C-terminus is important in the functional regulation of the M_1R, but not the membrane expression.

Keywords: muscarinic receptor, palmitoylation, molecular biology, electrophysiology, receptor activity

P11-34

TRPML3 trafficking from the plasma membrane is regulated by the endosomal SNARE VTI1B for autophagosome biogenesis

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TRPML3 is a Ca^{2+}-permeable cation channel that is expressed in multiple subcellular compartments including the plasma membrane as well as the endocytic and autophagic pathways. We have previously shown that upon induction of autophagy, TRPML3 traffics to the phagophore by palmitoylation, and Ca^{2+} release via TRPML3 as a downstream effector of phosphatidylinositol-3-phosphate at the phagophore is crucial for autophagosome formation. However, it is still not clear which compartment TRPML3 originates from in order to function in autophagy. Here, we show that TRPML3 interacts with an endosomal SNARE VTI1B that is involved in clathrin-mediated endocytosis. Indeed, TRPML3 was internalized upon autophagy stimulation, while accumulated at the plasma membrane by autophagy inhibition. Importantly, inhibition of the interaction between TRPML3 and VTI1B not only made TRPML3 retained at the plasma membrane but also suppressed autophagy. Antibody uptake assay revealed that TRPML3 at the plasma membrane truly translocates to the autophagosomes upon autophagy induction. However, the VTI1B non-binding mutant of TRPML3 showed decreased movement and reduced recruitment to the autophagosomes. Collectively, these data suggest that the Ca^{2+} channel TRPML3 is recruited from the plasma membrane to the autophagic structures upon induction of autophagy by interaction with VTI1B.

Keywords: TRPML3, autophagy, VTI1B, SNARE, trafficking

P11-35

Plasma membrane localization and function of the ABHD17 family

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Palmitoylation, the only reversible process of the post-translational protein lipidation, attaches palmitic acid to target protein cysteine via thioester bond. Approximately 11% of all human proteomes may undergo palmitoylation. Especially, diverse pathogenic proteins, including cancer, diabetes, arrhythmia, Schizophrenia, Huntington's disease, and Alzheimer's disease are palmitoylated, but only a few proteins defined the function of palmitoylation, making it palmitoylation entering a golden age as a research subject in this field. This process is mediated by a zDHHC-PATs, reversed by protein depalmitoylases, such as APT, PPT, and ABHD17. The most recently discovered ABHD17 family members (ABHD17A-C) directly remove palmitic acid from cysteine residues in target proteins, but its own properties and the exact molecular mechanisms underlying translocation to the PM remain uncertain. Here, we find that the palmitoylation codes orchestrate intracellular patterning of ABHD17 and middle region of cysteine cluster is critical for plasma membrane targeting and protein depalmitoylation. Moreover, hydrophobic amino acids adjacent to the cysteine cluster conduct an initial absorption to the endomembrane where PATs are present before palmitoylation of ABHD17 family. Interestingly, N-terminus alone fails to target the endosome, and palmitoylation of ABHD17 is properly achieved only when it utilizes the canonical endosomal targeting motif

in the alpha/beta hydrolase fold. Our findings demonstrate that there are three requirements for ABHD17 to bind to PM: (1) adsorption to the Golgi is mediated through hydrophobic residues in the N-terminus, (2) translocation to the endosome is facilitated by endosome targeting motif, and (3) palmitoylation occurs in the N-terminus. Collectively, this research unravels the underlying mechanisms governing the intricate interplay of ABHD17 family's subcellular localization, palmitoylation, and functional regulation.

Keywords: post-translational modification, palmitoylation, ABHD17, plasma membrane, lipidation

P11-36

Two specific interactions between TRPML3-GABARAPL2/GATE16-RAB33B regulate autophagy

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ATG8s are essential for autophagy as they recruit various autophagy machineries to the phagophore. We showed that the intracellular Ca^{2+} channel TRPML3 specifically interacts with the mammalian ATG8 homologue GABARAPL2/GATE16 but not with LC3B to increase autophagy. However, the mechanisms underlying the specificity and the role of the interaction are yet to be investigated. Here, we report that a single amino acid motif in ATG8s determines the specificity of the TRPML3-GATE16 interaction and that mutation of this motif reduces both the interaction and autophagy. Additionally, inhibition of the TRPML3-GATE16 interaction from the TRPML3 side also suppresses autophagy, suggesting the importance of this interaction in autophagy regulation. To further explore the role of the interaction, we searched for ATG8-related TRPML3 interacting proteins and discovered that RAB33B, a Golgi resident small GTPase contains a LC3-interacting region (LIR) motif. We found that RAB33B specifically interacts with GABARAPL2/GATE16 through LIR among the ATG8s and is recruited to the phagophore in a GATE16-dependent manner. Moreover, mutations in the LIR of RAB33B decrease the TRPML3-RAB33B interaction and autophagy. These results suggest that two specific interactions involving GABARAPL2/GATE16 play a crucial role in autophagy by recruiting TRPML3 and RAB33B and forming protein complexes at the phagophore to promote autophagosome formation.

Keywords: Autophagy, TRPML3, GABARAPL2/GATE16, RAB33B, LIR

P11-37

Single-molecule imaging to reveal a role of PI(4,5)P_2-channel interactions in M-channel trafficking regulation in neurons

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Background: In neurons, M-channel (Kv7.2/7.3) is one of the most important voltage-gated potassium channels to regulate action potential generation. Pathologically, since M-channel dysfunction is a cause of various neurological diseases including epilepsy, M-channel is therefore considered as an important drug target for these diseases. M-channel regulation mechanisms can be roughly divided into the following two mechanisms: trafficking and activity regulation. For the former, it has been well known that M-channels predominantly localize to the axon initial segment (AIS) and the plastic changes in the spatial pattern of M-channels are directly related to the neuronal excitability. For the latter, it has already been demonstrated that PI(4,5)P_2, a type of phosphoinositide in the inner leaflet of plasma membrane, electrostatically interacts with several basic residues of M-channel to stabilize its activated-open state. Although PI(4,5)P_2 is also strongly associated with vesicle formation and trafficking processes, no one has yet fully verified whether PI(4,5)P_2 regulates not only the activity but also the trafficking of M-channels in neurons.

Methods and Results: In this study, to clarify the role of PI(4,5)P_2-channel interactions in regulating M-channel trafficking, we investigated the subcellular localization of M-channel mutants lacking PI(4,5)P_2 binding ability in neurons. The results showed that the mutants were preferentially transported to the AIS similarly to wild-type M-channels, however the surface density of the mutants at AIS region was significantly reduced in a channel activity dependent manner. In addition, the more detailed trafficking processes of M-channels in neurons were detected and analyzed by using total internal reflection fluorescence microscopy (TIRFM) and machine learning. Specifically, we succeeded in distinguishing and quantifying the multi-modal 3D dynamics including lateral diffusion and exo/endocytosis of M-channels at a single molecule resolution. Interestingly, we found that the mutation of PI(4,5)P_2 binding site affected both lateral diffusion and exo/endocytosis processes of M-channels. In summary, this study presents a novel perspective suggesting that PI(4,5)P_2 plays a direct regulatory role not only in the activity, but also in the trafficking of M-channels.

Keywords: M-channel, PI(4,5)P2, single molecule imaging, axon initial segment, epilepsy

P11-38

In vivo mapping of subcellular proteomes in mice

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To facilitate the understanding of metabolic changes associated with disease, we have developed new in vivo tools that enable tissue-specific profiling of subcellular proteomes. First, we describe a method to profile in vivo mitochondrial proteomes utilizing transgenic mice expressing MTS-APEX2 (MAX-Tg), a peroxidase-based proximity labeling enzyme containing a mitochondrial matrix targeting sequence. Upon label activating conditions, mitoAPEX rapidly (<1 min) catalyzes production of biotin radicals which biotinylate proteins within a 20 nm radius. Mass analysis of biotinylated proteomes confirmed specific and efficient labeling of the mitochondrial proteome and revealed tissue-specific patterns of the matrix proteome. Of these, we newly identified that RTN4IP1 is localized to the mitochondrial matrix and regulate CoQ biosynthesis. Second, we also generated iSLET (in situ Secretory protein Labeling via ER-anchored TurboID) mice which labels secretory pathway proteins, via proximity labeling activity by ER lumen targeted TurboID, an engineered biotin ligase. We expressed iSLET in the mouse liver and demonstrate efficient labeling of the liver secreted proteome which could be tracked and identified within circulating blood plasma. We expect MAX-Tg and iSLET mice will facilitate our understanding of mitochondrial function and interorgan communication.

Keywords: proteome, ER, mitochondria, secretory protein, matrix

P11-39

Rapamycin and hydroxychloroquine impact in auranofin-treated lung cancer cells: apoptosis, ROS, and glutathione

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Auranofin, an inhibitor of thioredoxin reductase (TrxR), inhibits the growth of a variety of cancer cells. However, little is known about the toxicological effect of auranofin in human lung cancer cells in relation to autophagy. Here, we investigated the effects of Hydroxychloroquine (HQ; an inhibitor of late-stage autophagy) and Rapamycin (Rapa; an inducer of autophagy) on auranofin-treated Calu-6 and A549 lung cancer cells in relation to cell death, reactive oxygen species (ROS), and GSH levels. Auranofin inhibited the growth of Calu-6 and A549 cells with IC50 values of 3 μM and 6 μM at 24 h, respectively. Auranofin induced apoptosis and necrosis in these cell lines, which were accompanied by the loss of mitochondrial membrane potential (MMP; ΔΨm). ROS levels including O2 and GSH depletion were increased in auranofin-treated Calu-6 and A549 lung cancer cells. Auranofin promoted autophagosome accumulation by inducing early-stage autophagy but inhibited autophagic flux by blocking the fusion of autophagosome and lysosome, the step of late-stage autophagy, as evidenced by increases in LC3A/B form, ATG7, Beclin-1and P62 in auranofin-treated Calu-6 and A549 cells. Treatment with 20 μM HQ increased cell growth inhibition, MMP loss, ROS levels and GSH depletion in auranofin-treated Calu-6 and A549 cells. However, treatment with 200 nM Rapa slightly prevented apoptotic cell death, MMP loss and ROS levels. In conclusion, Auranofin induced the growth inhibition of A549 and Calu-6 cells via apoptosis and inhibition of autophagy flux. Rapamycin promoted auranofin-induced Calu-6 and A549 cell death by increasing O2, TrxR activity levels and depleting GSH. Hydroxychloroquine increased auranofin-induced Calu-6 and A549 cell death by increasing O2, TrxR activity levels and depleting GSH. The activation of autophagy might be a mechanism for preventing cell death and decreasing O2levels and antioxidant levels. [The present study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2019R1I1A2A01041209).]

Keywords: lung cancer, auranofin, apoptosis, ROS, glutathione

P11-40

The repurposed drug haloperidol induces apoptosis in neuroblastoma through the modulation of endoplasmic reticulum (ER) stress and autophagy

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Haloperidol, a typical antipsychotic drug, is used to treat schizophrenia. Although anticancer effects of various anti-psychotropic drugs have been reported as repurposing drugs, the anticancer effect of haloperiol in neuroblastoma has not been reported. In this study, we investigate the anti-tumour effect of haloperidol in neuroblastoma cell lines *in vitro* and *in vivo*. We found that haloperidol significantly inhibited cell proliferation in a dose dependent manner in SK-N-BE(2) and SK-N-SH neuroblastoma cells, but had no effect on non-cancerous HUVEC and CCD-1079SK cell lines. Haloperidol has been shown to increase apoptosis through nuclear condensation and loss of mitochondrial membrane potential, cell cycle arrest in the G2/M phase, and induction of ER stress activity. Haloperidol also inhibited the metastatic ability of SK-N-BE(2) and SK-N-SH cells. Furthermore, haloperidol (10 mg/kg) injection significantly inhibited xenograft tumor growth. These findings demonstrate that haloperidol may be useful in the development of anti-cancer agents for neuroblastoma treatment.

Keywords: neuroblastoma, haloperidol, apoptosis, mortality

P11-41

Rk-1 induces apoptotic cell death of human brain cancer through caspase 12-mediated regulation of ER stress

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Brain cancer, especially neuroblastoma and glioblastoma, are the most common solid cancers of the nervous system. Although the ginsenoside Rk1 has been shown to have anti-cancer effects against various cancers, its mechanisms against brain cancer are still poorly understood. In this study, we used SH-SY5Y cells and U87MG cells to investigate the underlying mechanisms of Rk-1 against human brain cancer cells. The results showed that RK-1 induced cell growth inhibition and apoptosis in a dose-dependent manner in both SH-SY5Y and U87MG cell lines. The pro-apoptotic genes Bak, caspase-9, cleaved (C)-PARP, C-PARP, and C-casp3 expression were increased, while the anti-apoptotic gene Bcl2 expression was downregulated. RK-1 also inhibited EMT, but induced MMP loss, ER stress activity, and activation of intracellular cellular calcium levels. Collectively, these results suggest that RK-1 induces apoptosis of human brain cancer cells through mitochondria-mediated intrinsic pathway and caspase 12-mediated regulation of ER stress. Therefore, RK-1 may be a promising candidate for the development of anticancer drugs targeting human brain cancer.

Keywords: ginsenoside RK-1, apoptosis, ER stress, brain cancer, neuroblastoma

P11-42

Etoposide-induced protein 2.4 homolog (EI24) regulates ferroptosis resistance in cancer

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Background: Ferroptosis is well-known as a nonapoptotic iron-dependent cell death that can be occurred in cancer by natural processes or synthetic agents. Targeting ferroptosis as cancer therapeutic strategies by understanding of ferroptosis-inducing and ferroptosis defense mechanisms in tumor suppression is essential for precision cancer medicine discovery in recent years. Recently, glutathione peroxidase 4 (GPX4) is one of the most important regulators of ferroptosis cancer cell death by the ability to scavenge the overaccumulation of lipid per-

oxide on not only cellular but also organelles membranes. Etoposide-induced protein 2.4 homolog (Ei24) showed various effects on cancers. However, the function of Ei24 in cancer research was still controversial. On breast cancer, Ei24 is working as tumor suppressor through apoptosis. Reversely, Ei24 promoted skin cancer progression via EGFR-PKCα interaction. Although there are many findings on Ei24 that have been published in recent years, there is a limitation in learning the role of Ei24 on ferroptosis regulation. Therefore, our study is focusing on the contribution of Ei24 to ferroptosis cancer cell death, especially on the connection between Ei24 and GPX4.

Results and Discussion: Our results showed that the overexpression of Ei24 rescued the cell viability of various cancer cell lines from ferroptosis inducer, Erastin, the system xC^- inhibitor. Whereas knockout of Ei24 consistently induced cancer cell death under Erastin treatment. Interestingly, we observed that Ei24 was positively correlated with GPX4 expression. Therefore, we examined how Ei24 can affect GPX4 during Erastin treatment. The results determined that Ei24 was involved in GPX4 translation process. Furthermore, Ei24-overexpressed cells upregulated AKT-mTOR-4EBP1 pathway.

Conclusion: In this study, we indicated the Ei24 novel function on ferroptosis resistance in cancer. Our study suggests that Ei24 is a tumor promoter and targeting Ei24 could be considered as a therapeutic target for cancer treatment therapy.

Keywords: Ei24, ferroptosis, cancer cell death, GPX4

P11-43

Cellular stress investigations and homeostasis mechanisms on fibroblast cultures regarding Hydrogen sulfide (H2S) used as a natural therapeutic factor in the Neuro-Myo-Arthro-Kinetic (NMAK) pathology

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The body's oxygen toxicity is generated by reactive oxygen species (ROS), which can have a beneficial or toxic effect depending on the type and concentration. The impact of ROS relies not only on the concentration of pro-oxidant molecules generated but also on tissue antioxidant reserves. ROS can function at physiological concentrations as secondary messengers and influence normal growth and division, stimulate DNA synthesis, and induce gene expression. In pathological conditions, an increase in ROS causes significant changes in cellular proteins and nucleic acids.

This study aims to investigate the cellular and molecular mechanisms underlying the therapeutic effects of natural therapeutic factors, specifically mud and sulfurous mineral waters. The investigation will be carried out by electrophoresis, ELISA, and Western blotting on primary fibroblast cultures obtained from Wistar rats. The two main physiological mechanisms investigated are inflammatory processes and the balance of oxidative stress, which are assumed to be influenced by mud and sulfurous natural mineral waters.

Previous scientific data show that different cell types are recruited during the inflammatory process, including fibroblasts, which respond to different intercellular and microenvironmental signals. This leads to the regulated production of various pro- and anti-inflammatory mediators, including cytokines such as tumor necrosis factor (TNF)-α and interleukins (IL)-1β and IL-6, chemokines, and enzymes such as cyclooxygenase (COX)-2.

The concept of oxidative stress caused by free radicals argues for the consideration of biomarkers of oxidative stress. The oxidative and reductive activity of enzymes acting on glutathione, thioredoxin, and other substrates of interest in the oxidation-reduction process reflects not only the level.

The study also examines the protective effect of H2S on neurons expressed against oxidative stress by increasing the substrate for producing the antioxidant GSH, including the cystine/glutamate antiporter and intracellular Cys concentrations. Furthermore, H2S has vasculoprotective properties in endothelial and vascular smooth muscle cells, such as triggering vasorelaxation and decreasing platelet aggregation. In addition, H2S possibly activates plasma membrane voltage-gated channels and mobilizes intracellular Ca^{2+} stores, providing neuroprotective effects.

In conclusion, this investigation sheds light on the cellular and molecular mechanisms underlying the therapeutic effects of natural therapeutic factors, specifically mud and sulfurous mineral waters, on inflammatory processes and oxidative stress. The findings of this study will serve as a scientific foundation for the therapeutic effects of these natural therapeutic factors.

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Keywords: hydrogen sulfide, Neuro-Myo-Arthro-Kinetic (NMAK) pathology, fibroblast cultures, reactive oxygen species (ROS), inflammatory process

P11-44

Effect of high glucose and ambient particulate matter on endothelial inflammation and its related mechanisms

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Epidemiological studies have revealed that ambient particulate matter (PM) and diabetes are risk factors for cardiovascular disease. However, people living in urban cities often encounter health issues concerning simultaneous exposure to air pollution and hyperglycemia. The combined effects on endothelial function and underlying mechanisms remain unclear. In the present study, we used human umbilical vein endothelial cells (HUVEC) to establish an *in vitro* model exposed to PM and high glucose, and to investigate whether PM and high glucose can affect mitochondrial ROS production, mitochondrial fission, mitophagy, and endothelial inflammation. The results have shown that PM aggravated levels of mitochondrial ROS, mitochondrial fission-related protein p-DRP1, autophagy-related protein p62 and LC3B, mitophagy-related protein BNIP3, and inflammation-related protein ICAM-1 in high glucose-treated HUVEC. Furthermore, to evaluate the relationship between mitochondrial function, mitochondrial fission, and mitophagy, several inhibitors targeting mitochondrial ROS (MitoQ), mitochondrial fission (Mdivi-1), or mitophagy (Bafilomycin A1) were used to delineate the upstream and downstream pathway underlying the endothelial inflammation. Based on these results, we thoroughly clarify that combined exposure to PM and hyperglycemia aggravate an endothelial injury and related regulatory mechanisms involving mitochondrial ROS, mitochondrial fission, mitophagy, and endothelial inflammation. These findings elucidate the molecular mechanisms for air pollution and diabetes-induced endothelial injury.

Keywords: PM2.5, high glucose, mitochondrial fission, mitophagy, endothelial inflammation

Poster

Poster

P11-45

Protective effects of intermittent hypoxia against hydrogen peroxide-induced oxidative stress in rat cerebellar astrocytes

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Increasing evidence indicated the protective effects of intermittent hypoxia (IH) preconditioning against ischemia/reperfusion or hypoxia/reoxygenation injury. Astrocytes, the most abundant subtype of glial cells in the central nervous system, provide physical and metabolic supports for neurons, and play an active role in neuronal repair. Previous studies showed that IH induces cell cycle arrest rather than cell death on rat cerebellar astrocytes. The aim of this study was to investigate the protective effects of IH on rat cerebellar astrocytes. Rat primary cerebellar astrocytes were cultured in an incubator with an oscillating O_2 concentration between 20% and 5% every 30 min for IH preconditioning. Fura-2 and Rhod-2 stainings were performed to analyze the cellular and mitochondrial calcium concentration, respectively. Real-time PCR and Western blot analysis were performed to detect the levels of antioxidant enzymes. IH slightly increased superoxide anion (O_2^-) with undisturbed calcium homeostasis and the intact of mitochondrial membrane potential. In addition, IH preconditioning protected astrocytes against hydrogen peroxide (H_2O_2)-induced cell death. Moreover, H_2O_2-induced calcium imbalance and mitochondrial membrane potential depolarization were attenuated by IH. Treatment with superoxide dismutase (SOD) to eliminate the O_2^- produced by IH can abolish the protective effects of IH on the calcium homeostasis and mitochondrial membrane potential. Furthermore, IH treatment up-regulated the levels of antioxidant enzymes Zn/CuSOD and MnSOD, which might be correlated with the protective effects of IH on astrocytes. Our findings suggest that IH protected astrocytes against the H_2O_2-induced oxidative stress through maintenance of calcium homeostasis, mitochondrial membrane potential and upregulation of antioxidant enzymes.

Keywords: astrocytes, intermittent hypoxia, hydrogen peroxide, oxidative stress

P11-46

In silico prediction and validation of PI3KCA as the target of novel miR-1133 in human umbilical vein endothelial cells exposed to hyperglycemia

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Background: Hyperglycemia, which is the hallmark of diabetes mellitus, is a major contributor of oxidative stress, apoptosis and diabetic vascular endothelial dysfunction. MicroRNAs play a role in the development of vascular endothelial dysfunction in diabetes. Our preliminary bioinformatics analysis on human umbilical vein endothelial cells (HUVECs) exposed to hyperglycemia showed an upregulation of novel miR-1133.
Objective: This study aimed to predict and validate the target genes of novel miR-1133 in HUVECs exposed to hyperglycemia.
Methodology: The target genes of novel miR-1133 were predicted via in silico analysis using miRDB prediction algorithm. The potential target gene list was narrowed down by selecting diabetes-, oxidative stress- and apoptosis-related genes and pathways using the DAVID bioinformatics to understand their overall functional themes and representation. The expression of novel miRNA-1133's target genes and protein were validated through quantitative polymerase chain reaction (qPCR) and western blot, respectively.
Results: Based on the analysis, a total of 132 target genes were predicted. Novel miR-1133 targeted phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PI3KCA) to regulate oxidative stress, apoptosis and diabetes in endothelial cells. Quantitative analysis of RNA-sequencing reads and subsequent validation by stem-loop qPCR confirmed that novel miR-1133 was upregulated in HUVECs exposed to hyperglycemia ($P < 0.01$). Meanwhile, the gene and protein expression of PI3KCA were decreased ($P < 0.05$).
Conclusion: Our results suggest that PI3KCA is the target gene of novel miR-1133, with potential modulation on oxidative stress and apoptosis in hyperglycemia-induced HUVECs. Thus, further functional study is required to validate the role of novel miR-1133 on the PI3KCA pathway, which could serve as potential therapeutic target for diabetic vascular endothelial dysfunction.

Keywords: diabetes, endothelial dysfunction, human umbilical vein endothelial cells, microRNA, oxidative stress

P11-47

EI24 benefits cancer DNA repair via regulating purine synthesis

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DNA repair pathways can enable tumor cells to survive DNA damage induced by chemotherapy and radiation treatments, leading to an escalating rate of acquired therapy resistance in cancer. However, the molecular mechanisms by which cancer promotes DNA repair and evades such damage have not been clearly understood. Etoposide-induced protein 2.4 homolog (EI24), located on the endoplasmic reticulum (ER) membrane, has been reported to be a stress sensor in certain stress conditions, particularly ER stress. Here, we hypothesize that EI24 also plays a role in sensing and regulating DNA damage stress. Using DNA-damaging drugs to treat a set of breast cancer cells, we observed that overexpression of EI24 correlated with rescued cell viability, suggesting EI24 protected cancer from DNA damage-induced cell death. Mechanistic studies revealed that EI24-overexpressing cells exhibited an enhanced and earlier response to DNA damage compared to control cells, which was evident by elevated γH2AX levels. Biochemical investigations demonstrated that adenosine, but not other nucleotides, was important for cancer cell survival under DNA damage, and that, interestingly, EI24 facilitated adenosine *de novo* synthesis by promoting the expression and activity of rate-limiting enzyme ADSS1. Such effect was more visual under DNA damage conditions, which efficiently provided adenosine for DNA damage repair process. EI24 regulated ADSS1 likely via protein translation, as shown by polysome fractionation analysis. Taken together, our findings establish a role of EI24 in conferring cancer resistance to chemotherapy and radiotherapy which suggests the com-

bination of purine synthesis inhibitors and DNA-damaging therapies as a promising strategy to combat resistance and improve cancer treatment.

Keywords: DNA damage, DNA repair, purine synthesis, ADSS1, chemo-radiation resistance

P11-48

Comparison of obesity and aging signatures in murine adipose tissue at single-cell resolution

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Obesity and aging are major risk factors for various diseases and thus represent heavy socioeconomic burden to global healthcare. The two processes share similar, if not convergent, phenotypes, and increasing evidence suggests that one accelerates the other. Both obesity and aging are associated with insulin resistance, chronic low-grade inflammation and dysfunction of systemic energy homeostasis. However, there are many differences as well and the molecular mechanisms behind such similarities and differences remain unclear. Senescent cells accumulate in both conditions, each of which can be ameliorated by their removal. Interestingly, senescent cells emerge particularly early on in adipose tissue of mice on high fat diet. Hence, senescent cells may serve as a bridge between obesity and aging for adipose tissue physiology. Many studies have characterized adipose tissue of lean and obese mice at single-cell level, but obese and aged adipose tissue have not been directly compared side-by-side. Here, we compare single-cell transcriptomics of epididymal white adipose tissue (eWAT) and inguinal WAT (iWAT) in lean, diet-induced obesity, and slightly more aged conditions. Clustering of adipose stem and progenitor cells (ASPCs) revealed depot-specific differences in previously reported cell subtypes. More cells were in a further differentiated, committed stage in eWAT than in iWAT. Interestingly, stress-unresponsive, quiescent-like cells accumulated in obese and aged tissues. The frequency of $p16^{INK4a}$-expressing senescent cells increased in both obese and aged tissues, although their absolute abundance was extremely low. Our comparative single-cell transcriptome analysis provides a detailed resource to investigate functional genomics behind obesity and aging.

Keywords: adipose, single-cell, aging, senescence, obesity

P11-49

The protective mechanism of TMEM16E-mediating micropinocytosis in plasma membrane repair (PMR) systems

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Transmembrane protein 16E (TMEM16E), a Ca^{2+}-activated phospholipid scramblase, regulates diverse biological functions including apoptosis and blood coagulation by altering the physical properties of the plasma membrane. Recent studies reported that expression of TMEM16E and TMEM16F belongs to TMEM16 family is important for plasma membrane repair (PMR) systems by resealing the damaged membrane. Here, we found that TMEM16E maintained membrane integrity by inducing the macropinocytosis of phospholipid-scrambled plasma membrane. Intracellular Ca^{2+} rise activates the scramblase activity of TMEM16E protein and induces the targeting of Annexin V (AV) to the phosphatidylserine on the extracellular surface of plasma membrane, which is important in triggering the apoptotic pathway. Surprisingly, our data shows the membrane-targeted AV-molecules were rapidly internalized to the cytoplasm region of cells expressing TMEM16E. The internalization of AV-molecules was almost completely inhibited by the application of amiloride, a macropinocytosis blocker, suggesting that the AV enters into the cells through the macropinocytosis pathway. Interestingly, we identified that the growth factor receptor (GFR), a macropinocytosis receptor directly interacts with TMEM16E, GFR was ligand-independently activated by the scrambling activity of TMEM16E. Consequently, macropinocytosis is caused through increased PI3K by GFR. Additionally, the internalization of AV-molecules contributed to maintaining the cell morphology. These data demonstrate that the TMEM16E-dependent macropinocytosis is important for cell survival by maintaining membrane integrity. Together, our data provide substantial evidence showing a novel protective mechanism of TMEM16E protein through the activation of scramblase-mediating macropinocytosis.

Keywords: TMEM16E, phospholipid scramblase, macropinocytosis, plasma membrane repair (PMR) systems, membrane integrity

P11-50

The role of TRPV4 channel in wound healing of murine esophageal keratinocytes

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Background: Transient receptor potential vanilloid 4 (TRPV4) is a non-selective cation channel that is widely expressed in different body tissues and plays several physiological roles. This channel is highly expressed in esophageal keratinocytes where its activation mediates ATP release. However, whether TRPV4 has a role in wound healing of esophageal keratinocytes is unclear. In this study, we investigated the putative role of TRPV4 in the migration and proliferation of esophageal keratinocytes.
Methods: Esophageal keratinocytes were isolated from wild-type (WT) and TRPV4-deficient (TRPV4-KO) mice, cultured and treated with various chemicals and agonists/antagonists. Wound healing assay was performed using cell culture inserts, and the closure of the cell-free gap was monitored using fluorescence microscopy. Time-lapse analysis was conducted to capture the healing process. Transfection experiments with TRPV4 cDNA were also conducted. Cell cycle assays and mechanical strain experiments were performed to assess cell proliferation. RT-PCR was used to analyze the mRNA transcription of different adenosine receptor subtypes, and immunostaining was performed to visualize cytokeratin 14 (CK14) expression by the keratinocytes. Statistical analyses were conducted using appropriate tests.
Results: Both cell migration and proliferation were slower in WT esophageal keratinocytes compared to TRPV4-KO cells. Exogenous ATP and NPPB (ATP release stimulant) had a stronger inhibitory effect on wound healing in WT cells compared to TRPV4-KO cells, which suggests that TRPV4-mediated release of ATP from esophageal keratinocytes contributes to a decrease in the rate of *in vitro* wound healing. The inability of apyrase (ATP hydrolase) to affect wound healing or negate the inhibi-

tory effect of exogenous ATP rules out a direct role for ATP in modulating wound healing. Therefore, we hypothesized that adenosine, as an ATP degradation product, could be a candidate molecule involved in modulating *in vitro* wound healing of esophageal keratinocytes. Our results clearly demonstrated the ability of exogenous adenosine to affect markedly and concentration-dependently wound healing in both WT and TRPV4-KO cultures. Our RT-PCR results showed that the A_{2B} receptor subtype had apparently high transcription level among the different subtypes in the esophageal mucosa, whereas genes encoding the A_1, A_{2A} and A_3 adenosine receptors were only weakly transcribed. These results are consistent with our findings, wherein a selective A_{2B}AR antagonist markedly enhanced wound healing in WT keratinocytes to levels that were comparable to TRPV4-KO cells, and significantly blocked the inhibitory effect of exogenous adenosine on gap closure in cultures of cells isolated from both mouse strains.
Conclusion: Our results suggest that TRPV4-mediated release of ATP from esophageal keratinocytes contributes to a decrease in the rate of *in vitro* wound healing via the ATP degradation product adenosine, which acts on A_{2B} adenosine receptors.

Keywords: cell cycle, esophagus, keratinocytes, TRPV4, wound healing

Poster

P11-51

Palmitoylation-mediated PHF2 loss aggravates hepatocellular carcinoma in the SREBP1c-dependent manner

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Background: Reprogramming in lipid metabolism is attracting increasing recognition as a hallmark of cancer cells. Palmitic acid (PA) is the most common fatty acid in the human body and also mediates palmitoylation. Numerous proteins are impacted by palmitoylation, but its pathophysiological functions are still poorly understood.
Methods: Palmitoylation of a tumor suppressor, plant homeodomain finger protein 2 (PHF2), was assessed using a liquid chromatography-mass spectrometry (LC-MS) analysis and an *in vitro* palmitoylation assay. Newly synthesized and elongated fatty acids in hepatocellular carcinoma (HCC) cells were monitored using stable 13C-labeled acetate with LC-MS. Each free fatty acid and cholesterol in cells were captured by gas chromatography-MS. The molecular target of PHF2 was found using LC-MS combined with functional enrichment analysis in HCC cells. The effects of PA and PHF2 on cell proliferation were determined using cell counting, colony formation, an oxygen-permeable 3D culture system, and a xenograft mouse model. Survival rates of HCC patients and the correlation between proteins were analyzed using the biopsied liver tissues from HCC patients.
Results: In this study, we demonstrated that PA acts as a molecular checkpoint for lipid reprogramming and cell proliferation in HCC cells. Mechanistically, a palmitoyltransferase, the zinc finger DHHC-type ZDHHC23 palmitoylates PHF2, which subsequently increases ubiquitin-dependent degradation of PHF2. This study also reveals that PHF2 acts as a tumor suppressor by exerting an E3 ubiquitin ligase activity toward sterol regulatory element-binding protein 1c (SREBP1c), a master transcription factor of lipogenesis. PHF2 degrades SREBP1c and reduces SREBP1c-dependent lipogenesis and cell proliferation. Notably, SREBP1c stimulates the production of free fatty acids in HCC cells and the consequent PA induction activates the PHF2/SREBP1c axis.
Conclusion: Overall, this study unravels a previously unexplored lipid reprogramming in HCC cells by PA/PHF2/SREBP1c axis. Since PA seems to be central to this axis, this study emphasizes the health risk associated with a PA-enriched diet regarding HCC progression.

Keywords: palmitic acid, PHF2, SREBP1c, proliferation, lipid metabolism

P11-52

Effect of fulvic acid derivatives on proliferation and differentiation of 3T3L1 adipocytes

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The risk of developing type 2 diabetes (T2DM) in obesity is as high as 80-85%. Various metabolic syndromes are also increased in obesity. Fulvic acid was found to alleviate the mean backfat thickness of pigs when it was supplied together with meals. However, the mechanism for the anti-fat accumulation effect of fulvic acid remains unclear. Therefore, we used 3T3-L1 adipocytes to explore the mechanism of fulvic acid. 3T3-L1 cells increased lipid accumulation on day 8 of differentiation and the lipid deposits were reduced by fulvic acid treatment. The expression of PPAR gamma and C/EBP alpha, which are proteins related to hyperplasia, decreased in 3T3-L1 cells treated with fulvic acid compared to control cells without fulvic acid treatment. In addition, expression of FABP4, CD36, and FAS, which are proteins associated with hypertrophy, more decreased in 3T3-L1 cells treated with fulvic acid. We found that this inhibitory effect of fulvic acid was not regulated by insulin, and the protein expression of adenosine monophosphate-activated protein kinase (AMPK) was increased by treating fulvic acid. These findings suggest that fulvic acid regulates the AMPK pathway, inhibiting the hypertrophy and hyperplasia of adipocytes. Regulating AMPK in 3T3-L1 adipocytes could be a therapeutic tool for obesity treatment.

Keywords: 3T3-L1, fulvic acid, AMPK, obesity

P11-53

Deficiency of IDH2 decreases brown adipocytes differentiation through inhibition of LncBATE10

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Brown adipose tissue (BAT), a crucial heat-generating organ, regulates whole-body energy metabolism by mediating thermogenesis. Isocitrate dehydrogenase 2 (IDH2) is an $NADP^+$-dependent enzyme that catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate in the mitochondrial matrix and is critical to produce NADPH. Here, we showed decrease of brown adipocyte differentiation in IDH2 knockout mice. Through RNA-seq analysis, we identified a brown adipose tissue-enriched long non-coding RNA (lncRNA), lncBATE10, that was required for a full brown fat differentiation. Moreover, the level of lncBATE10 was significantly decreased in the BAT of the IDH2 deficiency mice, accompanied by reduced expression of key genes involved in mitochondrial biogenesis, energy expenditure. Therefore, these data suggest that IDH2 deficiency may inhibit brown adipocytes differentiation by reduction of lncRNA-BATE10 gene expression.

Keywords: IDH2, brown adipocytes, differentiation, lncBATE10, brown adipose tissue

P11-54

Synergistic effects of lutein, zeaxanthin, and spearmint extracts against antioxidant stress in APRE-19 and HCE-T cells

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Vision protection has been one of the most popular topics in modern health care. The vision protection industry includes professional services by ophthalmologists and optometrists, and development in novel drugs and pharmaceuticals, eye glasses and contact lenses, vision aids and vision protection devices, and health food. Previous literature proposed that spearmint extract has beneficial effects for the mitigation of glaucoma, but it has not yet clarified whether lutein and zeaxanthin may be added to exert synergistic effects. In this study, ARPE-19 (human retinal pigment epithelial cells) and HCE-T (human cornea epithelial cells) were used to investigate the effects of various ingredient mixtures on the status of intracellular oxidative stress. For the study groups, different concentrations of lutein, zeaxanthin, spearmint extracts were pre-added to ARPE-19 and HCE-T cells, followed by exposure to ultraviolet B (UVB) irradiation. Parallel preparations without UVB irradiation were used as controls. All groups were subject to dichloro-dihydrofluorescein diacetate (DCFH-DA) assay, a quantitative method for oxidative stress assessment. The results showed that lutein or zeaxanthin alone can significantly reduce the oxidative stress of both cell lines, and spearmint extracts with lutein and zeaxanthin were also effective. These results will serve as the baseline data for development of these compound mixtures into vision protection products.

Keywords: lutein, zeaxanthin, spearmint extracts, APRE-19 cells, HCE-T cells

P11-55

A comparative study of nucleic acid and glycogen preservation in formalin-fixed and alternative fixative-fixed tissue sessions

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Formalin is a widely used fixative in histopathological practice because of its advantages: low cost, good fixation properties and compatibility with downstream histological applications. However, it is a potent irritant for the skin and nasal cavity and cytotoxic at high doses. As alternative, natural fixatives are proposed to substitute formalin in histopathological practice. Sugar-based fixatives provided promising results in preserving tissue structure; however, it has not reached application because of discrepancy in different studies. Moreover, very little information is available for histochemical staining.

Our study compares nucleic acid and glycogen preservation in sugar-based fixative- and formalin-fixed mouse liver samples using Methyl Green Pyronin Y (MGPY) and Periodic-acid Schiff (PAS), respectively. Fresh live tissues were collected from mice and fixed with formalin or raw cane sugar solutions (30%, 50% and 70%) for 24 hours. After routine tissue processing and sectioning, the mice's liver tissues were stained with Haematoxylin and Eosin (H&E), MGPY and PAS. 30% sugar solution produced fermentation smells after 24 hours of fixation. With H&E stain, 30% sugar solution-fixed tissues revealed cytoplasmic vacuolation suggestive of cell autolysis. 50% sugar solution-fixed tissues revealed the best tissue fixation and compactable with formalin-fixed tissues, while 70% sugar solution-fixed tissues showed tissue shrinkage with H&E stain. With MGPY stain, 30% and 50% of sugar solution-fixed tissues showed lesser staining density than formalin. 70% sugar solution-fixed tissues revealed proper staining of DNA and RNA.

Interestingly, the sugar solution could not preserve glycogen in liver tissue compared to formalin, even though collagen fiber staining was compatible with formalin by PAS stain. Our study concluded that sugar solution could replace formalin in small tissue samples, but the ideal concentration needs to be optimised for H&E and nucleic acid preservation. However, formalin is still superior to sugar solution in glycogen preservation of liver tissues.

Keywords: formalin, cancer prevention, tissue preservation, glycogen preservation, sugar based fixative

P12-1

Suncus murinus as a suitable model for studying daily torpor

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Torpor is a state of lowered body temperature due to active reduction of metabolic rate. Elucidation of the mechanisms of torpor may be useful because hypothermia has potential medical benefits in various species including humans. Mice have mainly been used in experiments aimed at clarifying the mechanisms of torpor. It has been shown that mice enter torpor only when confronted with food deprivation or a combination of food deprivation and exposure to a cold environment. Because these conditions are intricately intertwined, novel laboratory animals that enter torpor without imposing complex conditions are highly desirable for investigating the regulatory mechanisms of torpor. The purpose of this study was to establish a novel laboratory animal that enter torpor without imposing complex conditions. For this purpose, we focused on house musk shrews (Suncus murinus), since it has been reported that the shrews show an intermittent low body temperature. Body temperature was recorded by an implantable programmable temperature logger implanted subcutaneously in the mid-dorsal region between the shoulder blades. Torpor was defined as a drop in body temperature to below 30°C. When the shrews were kept at an ambient temperature of 24°C, most of the animals did not enter daily torpor. However, when the ambient temperature was lowered to below 20°C, all of the shrews showed torpor. The torpor was often observed between the end of the dark period and the beginning of the light period, indicating the possibility that onset of torpor is regulated by an endogenous circadian rhythm. Notably, torpor occurred in the shrews in the absence of a fasting procedure and/or short daylight condition. Hence, just putting the animals in a modestly cold environment with no other specific conditions is sufficient for inducing torpor in the experimental room. The shrews that were exposed to a stepwise decrease in ambient temperature from 24°C to 8°C entered torpor even after returning them to a room kept at 24°C. In conclusion, this study indicates that Suncus murinus may be a suitable model animal for elucidating the mechanism of daily torpor. Elucidation of the mechanisms of torpor by using this model may be useful for inducing a state of artificial hibernation in various species including humans.

Keywords: hypothermia, body temperature, shrew, cold acclimation, hibernation

P12-2

Association analysis of gut microbiota-metabolites-neuroendocrine changes in male rats acute exposure to simulated altitude of 5500m

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Hyperactivation of hypothalamic-pituitary-adrenal (HPA) axis and hypothalamic-pituitary-thyroid (HPT) axis were found in acute high altitude challenge, but the role of gut microbiota and metabolites is unknown. We utilized adult male Sprague-Dawley rats at a simulated altitude of 5500m for 3 days in a hypobaric-hypoxic chamber. ELISA and metabolomic analyses of serum and 16s rRNA analysis and metabolomic analyses of fecal samples were then performed. Compared with the normoxic group, serum corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), corticosterone (CORT), and thyroxine (tT_4) were increased in the hypoxia group, whereas thyrotropin-releasing hormone (TRH) was decreased. *Bacteroides*, *Lactobacillus*, *Parabacteroides*, *Butyricimonas*, *SMB53*, *Akkermansia*, *Phascolarctobacterium*, and *Aerococcus* were enriched in hypoxic group, whereas [*Prevotella*], *Prevotella*, *Kaistobacter*, *Salinibacterium*, and *Vogesella* were enriched in normoxic group. Metabolomic analysis indicated that acute hypoxia significantly affected fecal and serum lipid metabolism. In addition, we found 5 fecal metabolites may mediate the cross-talk between TRH, tT_4, and CORT with [*Prevotella*], *Kaistobacter*, *Parabacteroides*, and *Aerococcus*, and 6 serum metabolites may mediate the effect of TRH and tT_4 on [*Prevotella*] and *Kaistobacter* by causal mediation analysis. In conclusion, this study provides new evidence that key metabolites mediate the cross-talk between gut microbiota with HPA and HPT axis under acute hypobaric hypoxia challenge.

Keywords: hypobaric hypoxia, HPA axis, hypothalamic-pituitary-adrenal axis, HPT axis, hypothalamic-pituitary-thyroid axis, gut microbiota, metabolomics

P12-3

Cellular toxicology of carbon nanomaterials in environmental media

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Owing to their potential in a variety of application technologies, carbon nanomaterials such as fullerenes, carbon nanotubes, graphene sheets, and carbon quantum dots (CQDs) have inspired extensive research. However, the cytotoxic mechanism is poorly understood. Our research investigated the cytotoxic effect of carbon nanomaterials such as graphene oxide (GO), graphene oxide nanoribbons (GORs) and CQDs in vitro.

In the first study, the cytotoxicity of graphene oxide (GO) nanosheets with different morphologies regulated with Pb^{2+} was estimated using A549 cells. The results showed that after GO interacted with Pb^{2+}, GO could transform to graphite-like, tube-like, and ball-like structures. The cell viability significantly decreased when A549 cells were only exposed to GO; whilst more cells survived after exposure to GO regulated by Pb^{2+}. After aggregation and/or re-assembly, the membrane puncturing, phospholipid extraction, oxidative stress, nutrient depletion, and sheet adhesion of GO were inhibited to various extents, contributing to the reduced cytotoxicity of GO as well as Pb^{2+}.

Next, we investigated the cytotoxic effect of graphene oxide nanoribbons (GORs) on *Escherichia coli* (*E. coli*) in an in vitro method. The fabricated GORs formed long ribbons, 200 nm wide. We found that GORs significantly inhibited the growth and reproduction of *E. coli* in a concentration-dependent manner. GORs stimulated *E. coli* to secrete reactive oxygen species, which then oxidized and damaged the bacterial cell membrane. Moreover, interaction between GORs and *E. coli* cytomembrane resulted in polysaccharide adsorption by GORs and the release of lactic dehydrogenase. Furthermore, GORs effectively depleted the metal ions as nutrients in the culture medium by adsorption. Notably, mechanical cutting by GORs was not obvious, which is quite different from the case of graphene oxide sheets to *E. coli*.

Recently, the cytotoxicity and toxic mechanism of carbon quantum dots (CQDs) to *E. coli* were evaluated in vitro. The synthetic CQDs were extremely small in size (~2.08 nm) and displayed strong fluorescence. The results demonstrated that CQDs showed good biocompatibility with *E. coli* within a short culture time. However, when the exposure time exceeded 24 h, the toxicity of CQDs became apparent. On the one hand, the CQDs altered the surface charges of cells and induced lipid peroxidation by adhesion on the surface of *E. coli*, leading to an increase in the permeability of the cell wall. On the other hand, when the concentration of CQDs reached 200 μg/mL, the osmotic pressure of the extracellular environment was significantly reduced. These are the main factors that lead to cell edema and death.

In conclusion, the toxicity mechanisms of different carbon nanomaterials are different, and further evaluation of their toxicity is needed to provide theoretical basis for their future safe application.

Keywords: cellular toxicology, carbon nanomaterials, environmental media, graphene oxide, carbon quantum dots

P12-4

Effects of morphology regulated by Pb^{2+} on graphene oxide cytotoxicity: Spectroscopic and in vitro investigations

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In this study, the cytotoxicity of graphene oxide (GO) nanosheets with different morphologies regulated with Pb^{2+} was estimated using A549 cells and an in vitro method. The results showed that after GO interacted with Pb^{2+}, GO could transform to graphite-like, tube-like, and ball-like structures. The cell viability significantly decreased when A549 cells only exposed to GO (called GO group); whilst more cells survived after

exposure to GO regulated by Pb^{2+} (called GO + Pb group). The levels of reactive oxygen species obviously increased in all the groups of Pb, GO, and GO + Pb relative to the control group, which suggested all the exposure of Pb^{2+}, GO, and the mixture of GO and Pb^{2+} could significantly generate the oxidative stress-lipid peroxidization. GO showed the highest nutrient depletion-induced indirect cytotoxicity. In the GO + Pb group, the cell numbers got approximate 10% increase with respect to the GO group. Moreover, GO had high phospholipid extraction, whilst no phospholipid extraction was observed in the GO + Pb group. After aggregation and/or re-assembly, the membrane puncturing, phospholipid extraction, oxidative stress, nutrient depletion, and sheet adhesion of GO were inhibited to various extent, contributing to the reduced cytotoxicity of GO as well as Pb^{2+}.

Keywords: graphene oxide, morphology, lead(II), cytotoxicity, A549 cells

P12-5

Relationship between microgravity and myoblast proliferation

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Background: Microgravity induces muscle atrophy due to decreased protein synthesis and increased protein degradation in muscle, which is one of the most serious physical problems when going to space and needs to be solved. Although many previous studies have focused on the myoblast differentiation under microgravity, the only report focusing on proliferation (Tatiana et al. FASEB J. 2013) indicated that microgravity inhibited myoblast proliferation through decreased TRPC1 expression. However, the relationship between the suppression of myoblast proliferation and intracellular Ca^{2+} levels under microgravity remains unclear. Therefore, we hypothesized that microgravity would inhibit the calcineurin/NFAT pathway due to a decrease in the intracellular Ca^{2+} levels and then delay the cell cycle and cell proliferation. The purpose of this study was to test our hypotheses, and to identify the intracellular Ca^{2+} dynamics-related factors that are altered by microgravity.
Methods: The mouse skeletal muscle-derived cell line C2C12 cells were divided into two groups: a control group cultured under a 1G condition (CON group: n=6/time point) and a microgravity group cultured under a microgravity condition using the gravity control device Gravite® (MG group: n=6/time point). Cell numbers were measured at 24 and 48 hours. Intracellular calcium levels were examined using Fluo4-AM at 24 and 48 hours and quantified their brightness using Image J. We also investigated the gene expression levels of the cell cycle markers (Ki67, Cyclin, Cdk), intracellular Ca^{2+} dynamics-related factors on the sarcolemma (TRPC1, DHPR, Orai1, Piezo1), and on the endoplasmic reticulum membrane (RyR1, STIM1, SERCA1), and factors of the calcineurin/NFAT pathway (calmodulin, calcineurin, NFATc1) by quantitative RT-PCR analyses at 24 and 48 hours.
Results: Cell numbers at 24 and 48 hours were 0.69 and 0.58 times significantly fewer in the MG group than in the CON group, respectively. Intracellular calcium levels at 24 and 48 hours were also 0.50 and 0.72 times significantly decreased in the MG group than in the CON group, respectively. The gene expression levels of Ki67 and CyclinD1 mRNAs were decreased in the MG group at 24 and 48 hours, and STIM1, Piezo1, and calmodulin mRNAs were also decreased in the MG group at 24 hours. On the other hand, there was no change in the expression levels of TRPC1 mRNA. DHPR, RyR1, and SERCA1 mRNAs were not clearly detectable in C2C12 myoblasts to yield results.
Conclusion: Our results suggested that microgravity suppressed myoblast proliferation due to a decrease in intracellular Ca^{2+} levels during the early phase. The decreased expression of calmodulin and CyclinD1 indicates that the cell cycle was delayed by suppression of the calcineurin/NFAT pathway, and that the intracellular Ca^{2+} levels may be reduced through the decreased expression of STIM1 and Piezo1 mRNAs. Further detailed studies are needed to determine how microgravity suppresses intracellular Ca^{2+} levels in myoblasts.

Keywords: skeletal muscle, microgravity, calcium, myoblast, proliferation

P12-6

Exertional heat stroke causes neurobehavioral disorders in rats via modulating gut microbiota composition, gut barrier permeability, and systemic inflammation

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Background: Exertional heat stroke (EHS) is a severe medical condition characterized by increased body temperature due to strenuous physical activity in a hot environment. Mounting preclinical evidence suggests that gut microbiota can modulate brain development, function, and behavior by immune, endocrine, and neural pathways. This study aims to investigate whether the altered production of cytokines due to microbiota dysbiosis and subsequent systemic inflammation could potentially impact cognitive function in rats with EHS.
Methods: Wistar rats were randomized and divided into normal control (NC) and EHS groups. Adult male Wistar rats underwent forced treadmill running for 53 minutes in a 36°C/ 50% relative humidity until EHS onset, characterized by hyperthermia (42.8±0.3°C) and neurological dysfunction. All rats that were followed for 14 days survived. The feces and blood samples were collected for microbiota and cytokines/chemokines analysis before EHS and 3 and 14 days after EHS onset. Cognitive and motor function tests included modified neurological severity score (mNSS), radial maze, passive avoidance, and rotarod were performed. The histopathology of the brain and intestines was also performed. Correlations of neurobehavioral parameters and microbiota or cytokines were analyzed using Spearman's correlation coefficient.
Results: Three to fourteen days post-EHS onset, rats displayed neurobehavioral disorders, including cognitive deficits, increased inflammatory cell infiltration in the intestinal mucosa, intestinal hyperpermeability, and increased serum levels of pro- or anti-inflammatory cytokines/chemokines (e.g., IL-1, IL-10, thymus chemokines, MIP-1 alpha, and fractalkine). The feces sequenced by 16S rRNA high-throughput sequencing revealed four flora were significantly decreased after EHS time-dependently, including *Faecalibacterium*, *Prevotellaceae*, *Ruminococcaceae*, and *Prevotellaceae*. Sixteen flora were increased after EHS time-dependently, particularly in *Muribaculaceae*, *Prevotellaceae*, *Helicobacteraceae*, *Enterococcaceae*, *Lactobacillaceae*, *Lachnospiraceae*, and *Ruminococcaceae*, which were positively correlated with pro-inflammatory cytokines/chemokines expression and neurobehavioral deficits.
Conclusion: Our data suggest that EHS can cause neurobehavioral disorders in rats via modulating gut microbiota composition, gut permeability, and systemic inflammation.

Poster

Keywords: exertional heat stroke, microbiota, systemic inflammation, neurobehavioral deficits

P12-7

Hyperbaric oxygen therapy as a therapeutic approach for attenuating lung inflammation and restoring lung function following carbon monoxide poisoning

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Background: Carbon monoxide poisoning (COP) is a significant public health concern worldwide. Due to underreporting, the exact global burden of COP-induced pulmonary injury is challenging to determine. Therefore, preclinical and clinical studies should be conducted to elucidate the long-term respiratory consequences of COP, including the risk of developing chronic obstructive pulmonary disease, pulmonary fibrosis, or other respiratory complications. Hyperbaric oxygen therapy (HBOT) is considered the primary and most effective treatment for carbon monoxide poisoning. Whether HBOT attenuated the respiratory dysfunction caused by COP is still unknown. This study aims to investigate the long-lasting effects of COP on lung health and its underlying molecular and cellular mechanisms by using the national population-based cohort study and a rodent model. Furthermore, the effects of HBOT on lung function recovery caused by COP was also investigated.
Methods: In the preclinical study, Wistar rats were exposed to 1000 ppm CO for 20 min and further to 3000 ppm for another 3000 ppm. Long-term repeated intermittent HBOT was utilized as a treatment strategy. The lung parenchyma and BALF were harvested on the indicated day to evaluate the relevance of inflammatory assay and immune cell proportion. The pulmonary function was then estimated via the Flexivent system. For the epidemiological study, we identified patients from the National Health Insurance Database of Taiwan with COP (COP cohort) diagnosed between 1999 and 2017 and compared them with patients without COP (non-COP cohort) matched by age and the index date at a 1:3 ratio. We followed up the participants until 2018 to compare the development risk of COPD by using Cox proportional hazards regressions.
Results: Following CO inhalation, COP rats displayed abnormal behavior and histopathological deficits, increasing 14 kinds of cytokines/chemokines and over-activated immune cells in the BALF. HBOT could inverse the insults and downregulate 5 those of inflammatory mediators as well as inhibit $CD86^{+}$ and $CD163^{+}$ macrophage except for neutrophils. Lung epithelium showed hyperpermeability by losing tight junction protein cldn3 and ZO-1 except for cldn18, mitochondria and lamellar body disruption, and cell death via pyroptosis, and HBOT rescued the protein lost and prevented cells toward death. CO intoxication induced pulmonary dysfunction and exhibited chronic obstructive pulmonary disease (COPD)-like features at the late stage while HBOT reversed that impairment. For the epidemiological study, the COP cohort had a higher risk of developing COP than participants in the non-COP cohort.
Conclusion: We revealed the pathogenesis of lung injury under COP, ultimately developing COPD syndromes, and this finding was also consistent with epidemiology observation. We also demonstrated that HBOT protects against COP-induced lung dysfunction by attenuating lung inflammation and maintaining the alveolar epithelium integrity.

Keywords: carbon monoxide poisoning, lung injury, immune response, Chronic obstructive pulmonary disease, Hyperbaric oxygen therapy

P12-8

The effects of gestational and lactational exposure to cadmium on neurodevelopment

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Cadmium, an environmental hazard, can be easily exposed to daily life through diet and smoking.
Particularly, Cd can be transmitted to fetuses and infants through the placenta and breast milk, potentially impacting neurodevelopment. Cadmium is known to be associated with ADHD and blood cadmium concentrations.
However, the effects of low-level Cd exposure on neurodevelopment remain poorly understood. Therefore, we aimed to investigate the impact of low-level Cd exposure on neurodevelopment by exposing mice to Cd during pregnancy and lactation. The number of excitatory synapses between neurons from primary cultured neurons of PND 1 mouse exposed to low concentration of cadmium prenatally were significantly reduced. Furthermore, in the morphological development of neurons low-level cadmium exposure decreased neurite outgrowth, soma size and the number of branches. To assess the effects of low-level Cd exposure during pregnancy and lactation, brain tissues were collected from postnatal day 28 (PND 28) mice. As observed with Cresyl violet and Fluoro Jade B staining, low-level cadmium exposure during pregnancy and lactation resulted in a significant decrease in neuronal cells in the hippocampus of PND 28 mice without causing cellular toxicity. Furthermore, low-level Cd exposure induced abnormal cell proliferation and inhibited neuronal cell differentiation in the dentate gyrus of the mouse hippocampus. qPCR array analysis revealed upregulation of proinflammatory cytokines, microglia expression, and glutamate related factors in response to Cd exposure during pregnancy and lactation. Moreover, the intensity of GLT-1 and GFAP in brain tissues was increased by low-level Cd exposure. In an in vitro model, Cd-treated groups exhibited increased intensity of mGluR5 and GLT-1, along with a decreased punctate pattern of PSD95. Treatment with inhibitors of mGluR5 or GLT-1 decreased the intensity of mGluR5 and GLT1 and increased the punctate pattern of PSD95. The tendency of delayed synapse development induced by Cd exposure was mitigated by the inhibitors. Our findings suggest that impaired neurogenesis, synapse loss and delayed neuronal morphological development induced by Cd may be mediated through the overexpression of mGluR5 and GLT1.

Keywords: cadmium, neurodevelopment, synapse development, glutamate receptor, glutamate transporter

P12-9

A follow-up survey on the process of high-altitude A

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Background: High altitude acclimatization has been long-time studied. The aim of the present study is to track and record the changes in related physiological indicators during the process of high altitude ac-

Poster

climatization among lowlanders.
Methods: Using the method of cluster sampling, a follow-up survey on the process of high altitude acclimatization was conducted among new arrived Han lowlanders in Tibet University Medical College. A total of 107 Han Chinese immigrants participated. Hemoglobin concentration (Hb), oxygen saturation and heart rate were measured on the day of arrival and approximately once a week thereafter. Totally, 11 times measurements were recorded.
Results: There were differences in oxygen saturation and heart rate, while no difference was found in Hb between the Tibetan and Han ethnic groups at the first test day. Hb showed an increasing trend and reached its peak on the ninth test day, afterwards it began to decline. Moreover, oxygen saturation and heart rate showed a gradually increasing and decreasing trend, respectively.
Conclusion: Although several physiological indicators showed a trend of change with the extension of living time at high altitude, all indicators were within the normal range during the 4-month follow-up period after Han students arrived at the plateau. Further follow-up investigation will continue.

Keywords: altitude, acclimatization, hemoglobin, Han lowlanders, Tibet

P12-10

Element rich area as environmental cues affect human body accumulation of potentially toxic elements and their health

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Environmental effects and human health are the main topics of geomedical science. Human acculturation of these factors reflects the abundance of those elements in a given geographic area. The objective of this research is to explain the connection between geologic element concentrations and body accumulations as well as symptoms connected to specific elements. Geologically, Gorontalo Province has naturally high copper content, and anthropogenic, the environment is polluted by mercury from Artisanal and Small-scale Gold Mining (ASGM) activity. Examination of the geogenic elements (dust and soil) and hair samples from local people using Particle-induced X-ray Emission (PIXE) to see the relationship between geogenic elements and their accumulation in the human body. Results show that potentially toxic elements' accumulation in the human body follows the abundance of these elements in the geographic area, which then affect health and manifest with specific signs and symptoms. East Tulabolo is an area rich in copper (hazard quotient (HQ) in dust = 152.8), and most of the population shows the sign of Kayser–Fleischer rings. Likewise, the Dunggilata area has the highest concentration of mercury, especially in the dust (HQ = 11.1), related to ASGM activity in residential areas. Human hair is composed of keratin, a protein that forms the structural component of hair fibers. Geogenic elements can accumulate in hair through several pathways: direct absorption, dietary intake, and systemic circulation. These pathway same as like accumulation in human eyes. This study concludes that the geogenic concentration of elements parallels the accumulation of human tissue and manifests with element-related signs and symptoms.

Keywords: geomedical science, Copper, Mercury, ASGM, Gorontalo

P12-11

The acclimatization of Haenyeo to a cold environment and occupational characteristics evaluated by orexin and irisin levels

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Haenyeo is a woman who has the job of collecting seafood in the Jeju Sea at an average temperature of 13–14°C. The purpose of this study was to examine the cold acclimatization and occupational characteristics of Haenyeo through biomarkers such as orexin and irisin related to heat generation in the body.
A group of 21 Haenyeo and 25 general public group participants with similar ages and body mass indexes were randomly selected. In the cold-loading experiment, an automated climate chamber was set to 5°C and both feet were immersed in a 15°C water tank for 30 minutes. Tympanic temperature (T_{ty}) and skin temperature (T_{sk}) were measured, and the mean body temperature (mTb) was calculated. Blood samples were collected before and immediately after the examination. Orexin and irisin levels were analyzed.
Orexin levels were elevated after cold stimulation from 12.17 ± 4.44 to 12.75 ± 4.65 ng/mL (Haenyeo group, $P < 0.001$) and 10.37 ± 3.84 to 11.31 ± 4.02 ng/mL (control group, $P < 0.001$). Irisin levels were elevated after cold stimulation from 4.83 ± 2.28 to 5.16 ± 2.23 ng/mL Haenyeo group ($P < 0.001$) and control group ($P < 0.001$).
Our experimental results suggest that Haenyeo were superior in heat generation compared to the control group in conditions of chronic low-temperature exposure. This study increases the empirical reliability of the global agenda on climate change and suggests that it is necessary to establish a clinical foundation in the field.

Keywords: haenyeo, elderly women diver, brown adipose tissue (BAT), orexin, Irisin

P12-12

Mechanisms of peripheral sudomotor sensitivity to acetylcholine in endurance humans: Focused on activated sweat gland density

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The aim of this study is to identify the mechanism of peripheral sudomotor sensitivity in acetylcholine endurance humans. The study was conducted with long-distance runners(with 7-12 years of athletic training, average 9.262.1 years) to investigate the mechanism of peripheral nervous system (PNS) in endurance humans than the average humans. To this end, quantitative sudomotor axon reflex testing (QSART) with iontophoresis (2 mA for 5 min) and 10% acetylcholine (ACh) were performed to determine axon reflex mediated and directly activated (DIR, muscarinic receptor) sweating. In addition, Sweat onset time, sweat

rate, number of activated sweat glands, sweat output per gland and skin temperature were measured at rest while maximum oxygen uptake (VO2max) were measured during maximal cycling. Correlation was analyzed based on the collected data.

Keywords: quantitative sudomotor axon reflex testing (QSART), acetylcholine (ACh), long-distance runner, peripheral nervous system (PNS)

P13-1

Moxibustion improves hypothalamus *Aqp4* polarization in APP/PS1 mice: Evidence from spatial transcriptomics

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Aquaporin-4 (AQP4) is highly polarized to perivascular astrocytic endfeet. Loss of AQP4 polarization is associated with many diseases. In Alzheimer's disease (AD), it is found that AQP4 loos its normal location and thus reduce the clearance of amyloid-β plaques and Tau protein. Clinical and experimental studies show that moxibustion can improve the learning and memory abilities of AD. In order to explore whether moxibustion can affect the polarization of AQP4 around blood brain barrier (BBB), we used spatial transcriptomics (ST) to analyze the expression and polarization of *Aqp4* in wild type mice, APP/PS1 mice and APP/PS1 mice intervened by moxibustion. The results showed that moxibustion improved the loss of abnormal polarization of AQP4 in APP/PS1 mice, especially in the hypothalamic BBB. Besides, there are other 31 genes with *Aqp4* as the core have the similar depolarization in APP/PS1 mice, most of which are also membrane proteins. The majority of them have been reversed by moxibustion. At the same time, we employed the cerebrospinal fluid circulation gene set, which was found being on a higher level in the group of APP/PS1 mice with moxibustion treatment. Finally, in order to further explore its mechanism, we analyzed the mitochondrial respiratory chain complex enzymes closely related to energy metabolism, and found that moxibustion can significantly increase the expression of mitochondrial respiratory chain enzymes such as Cox6a2 in the hypothalamus, which could provide energy for mRNA transport. Our research shows that increasing the polarization of hypothalamic *Aqp4* through mitochondrial energy supply may be an important target for moxibustion to improve APP/PS1 mice's cognitive impairment.

Keywords: moxibustion, Alzheimer's disease, spatial transcriptomics, aquaporin-4, mitochondrial respiratory chain

P13-2

Can homeostasis of peripheral blood components alone shape the metabolic zonation in hepatic lipid metabolism?: A simulation study

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It has been reported that hepatocytes have gene expressions regiospecifically in the hepatic lobule and metabolize heterogeneously across sinusoids, a phenomenon termed metabolic zonation. Metabolic zonation is assumed to contribute to key liver functions, treated as an example of evolutionary optimization of metabolic function. If the assumption is correct, the extant spatial map of gene expressions across sinusoids would be optimized by some evolutionary constraints. In this study, we verified whether the homeostasis of peripheral blood components could be regarded as an evolutionary constraint. By using a mathematical model mainly representing hepatic lipid metabolism and bayesian optimization, we explored a variety of spatial gene expression maps that differ from the map found in the actual human liver. As a result, we found that about a thousand spatial maps of gene expressions can maintain the homeostasis of peripheral blood components. This result suggests that the homeostasis of peripheral blood components alone is not enough to shape the actual spatial map of gene expressions contributed to metabolic zonation. Therefore, there would be additional constraints besides the homeostasis of blood components. We will explore the additional evolutionary constraints shaping actual metabolic zonation by machine learning and mathematical optimization in the future.

Keywords: homeostasis, evolution, liver metabolic zonation, lipid metabolism, mathematical model

P13-3

Extracellular vesicle proteomics reveals the biochemical mechanisms of carbon monoxide poisoning-induced cardiac damage

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Background: Carbon monoxide poisoning (COP) can lead to severe cardiac complications, including myocardial ischemia, arrhythmias, inflammation, apoptosis, and endothelial dysfunction, all contributing to long-term cardiac sequelae such as heart failure and chronic heart disease. There is no biomarker has been universally accepted or routinely used in a clinical setting that could predict the long-term consequences of cardiac dysfunction caused by COP. Extracellular vesicles (EVs) and their cargo (proteins, lipids, RNA) reflect the condition of the cells they originated from. It's not clear which specific components of cardiac EVs might serve as effective biomarkers for CO-induced cardiac dysfunction. This study aims to investigate the association between cardiac EV proteomics and cardiac functions at day 28 following poisoning, utilizing an animal model of COP.
Methods: Echocardiography, blood levels of cardiac damage markers, and histology assay were used to determine COP-induced cardiac injury. Nanoparticle Tracking Analysis (NTA) and Transmission Electron Microscopy (TEM) were used to characterize cardiac EVs. The proteomics of cardiac EVs was analyzed using nano-scale liquid chromatography-tandem mass spectrometry (nLC-MS/MS).
Results: On day 28 after COP, the echocardiography and histology analysis revealed cardiac dysfunction and myocardium damage. In contrast, blood levels of cardiac markers (LDH, CK, and Troponin I) were elevated from day 7 to day 14 but not on day 28 of post-COP. Characterization of EVs revealed predominantly small EVs. Mass spectrometry proteomic analysis (nLC-MS/MS) demonstrated that some proteins were significantly altered >1.5 fold (down-regulated: 234, up-regulated: 39; $P<0.05$) at day 28 after COP, and more than half were restored by HBO treatment (down: 221/234, up: 21/39), suggesting that they are involved in the pathological process. Functional enrichment analysis associated these

proteins mainly with ribosomes, mitochondria, and sarcoplasmic reticulum. Among them, the abnormal expression of 17 proteins indicated the decreased ATP biosynthesis rate and the weird release & circulation of calcium ions at the sarcoplasmic reticulum in cardiomyocytes after COP injury, which may lead to cardiac insufficiency.
Conclusion: Our findings indicate that alterations in cardiac EV proteomics can effectively reflect the long-term cardiac consequences of COP. These changes hold promise as diagnostic biomarkers representing the prognosis of cardiac injury in individuals affected by COP.

Keywords: carbon monoxide poisoning (COP), cardiac dysfunction, cardiac extracellular vesicles (cardiac EVs), proteomics

P13-4

Massively parallel reporter assay for fine-mapping of NAFLD-specific expression quantitative trait locus

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Massively Parallel Reporter Assay (MPRA) is a high-throughput approach that allows the simultaneous determination of regulatory effects from thousands of non-coding sequences. To investigate the regulatory activity of NAFLD-specific expression quantitative trait loci (eQTL), we subjected 40 controls, 1107 LD-expanded eQTL SNPs, and 93 negative SNPs from NAFLD-eQTL dataset for MPRA analysis.
A total of 7,500 candidate sequences were synthesized and cloned into a reporter construct. The resulting library was transfected into HepG2 cells and the cells were subsequently treated with oleic acid/palmitic acid, thapsigargin, and vehicle to model fatty-liver associated stress. After sample collection, mRNA was extracted, and 3' tags were sequenced to determine which candidate elements carried regulatory effects and allelic enhancer activity.
Comparing tags from the library and mRNA revealed that more than 60% of the tested sequences have activity as enhancers. To validate the MPRA signals, we utilized ENCODE cCRE data and sequence-based prediction models, which showed a marginal enrichment in sequences with regulatory activity. This difference in enrichment may be attributed to the distinct nature of MPRA and other regulatory activity prediction models.
Integrative analysis for MPRA signals will be investigated for fine-mapping NAFLD-eQTL signals and find noncoding variants for regulating NAFLD-related traits.

Keywords: MPRA, NAFLD, liver, genomics, enhancer

P13-5

Characterization of differences in brain development in Atypical Rett syndrome mouse models

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Rett syndrome (RTT) is a rare genetic neurological disorder that severely impairs brain development, leading to mental and physical disabilities. Although individuals with RTT initially exhibit normal development during the first 6 to 18 months of life, they subsequently undergo a gradual regression, losing acquired linguistic, cognitive, and motor skills. While more than 90% of RTT cases come from de novo loss-of-function mutations in *MECP2*, there are patients with RTT-like phenotype but without mutation in *MECP2*. These atypical RTT patients harbor mutations in various genes that are relevant to brain development, including a GABA receptor *GABBR2*. Two specific mutations in *GABBR2* were frequently observed in atypical RTT patients. To explore the effects of these mutations, we generated *Gabbr2* mouse models carrying the mutations that were seen in human patients. Using the mice, along with *Mecp2* mice as positive controls, brain tissues were collected and were subjected to single-cell RNA sequencing. The subsequent analyses revealed the impact of *Gabbr2* mutations on brain development and identified the genetic pathways that can explain symptoms of typical Rett syndrome. Our findings provide valuable insights into the neurodevelopmental processes by the *GABBR2* mutations and the discovery of potential therapeutic targets.

Keywords: Rett Syndrome, Neurodevelopment, GABBR2, single nucleus transcriptomics, MECP2

P13-6

Genetic dissection of Hirschsprung's disease pathophysiology

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Hirschsprung's disease (HD) is a rare disease characterized by intestinal motility defect and obstruction due to the congenital absence of ganglion cells in the enteric nervous system. The incidence of HD is approximately 1 in 5,000 live births and can sometimes coexist with other syndromes, such as Down syndrome. The proportion and length of ganglion cells, as well as clinical severity vary among HD cases. The underlying cause of HD is known to be associated with the migration of neural crest cells. Genetically, although variants in the *RET* gene have been identified through genome-wide association studies, our understanding of the heritability and pathogenesis of the HD remain unclear. To investigate the genetic mechanism and its cellular consequences underlying development of HD, we performed single-nuclear RNA-seq (snRNA-seq) of 11 affected and 4 unaffected colon tissues followed by whole genome sequencing of 47 HD patients. Through this approach, we identified known genetic mutations associated with HD and other pathogenic mutations, which may drive expression of genetic pathways that are differentially regulated between patient groups of different genotypes. These findings provide valuable insights into the understanding of HD and have the potential to contribute to the development of future treatment strategies.

Keywords: Hirschsprung's disease, GI motility, snRNA-seq, gene regulation

P13-7

Characteristics of globin gene mutations in beta-thalassemia major patients with iron overload: A study from the Thalassemia community in East Java

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Background: Beta thalassemia major is a hereditary anemia, and Indonesia is one of the countries at risk for thalassemia. Thalassemia patients are known to have many characteristics of globin gene mutations, which have given rise to various debates about the emergence of various clinical manifestations and also the degree of severity. We conducted a descriptive study on the characteristics of globin gene mutations with clinical symptoms found in the thalassemia community in East Java.
Methods: We conducted a purposive sampling of 34 patients in the community to see the imaging of the globin gene mutation and the clinical imaging that appeared along with their ferritin levels.
Results: Of the 34 patients, 29.41% showed a genetic mutation of HbE(Cd26) and an unknown mutation location, then 47.06% had mutations of HbE/IVS-1-5, and 23.53% had mutations of HbE/Cd35. The mean age of the patients was 22 years, with a mean ferritin level of 1017 mcg/L. Of the three groups of mutations found in HbE and unknown mutation locations, they had the lowest average height (150 cm), the highest ferritin levels (1093 mcg/L), and lower Hb (8.3 g/dl).
Conclusion: In the thalassemia community in East Java, 3 HbE mutations were found, namely Cd 26 and unknown mutation location, Cd 26 and IVS-1-5, and Cd 26 and Cd 35. Mutations in the Cd26 globin gene and unknown mutation locations are known to have the characteristics of being shorter, more anemic, and having higher ferritin levels.

Keywords: mutation type, thalassemia, iron overload, clinical manifestations, ferritin

P13-8

Estimation of parameter probability distributions of Ion channel models based on bayesian inference using a Markov chain Monte Carlo method

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The properties of ion channels measured in experiments often exhibit wide variations, as evidenced by high variances in measurements. These variations can even occur among clones of the same genotype due to the instability and fragility of ion channels.
To model the gating mechanisms of ion channels, a common method is the continuous-time aggregated Markov model. This model represents each observable open and closed state with multiple states. To determine parameter values for such a Markov model, a widely used conventional method is point estimation from experimental measurements. However, point-estimated parameter values can be subject to uncertainty due to the high variances in measurements. Since the properties of an ion channel are not identical among individual clones in a series of experiments, a parameter of a Markov model should not have a certain value but rather a probability distribution.
This study introduces a method for estimating probability distributions of model parameters from experimental measurements based on Bayesian statistics. The probability of a set of model parameter values for the observed data is computed as the posterior probability using the likelihood of the parameters through Bayesian inference. Parameter sets are sampled according to the posterior probability using a Markov chain Monte Carlo (MCMC) method. Global sampling in the parameter space is achieved using replica exchange MCMC sampling, where samples by tempered replicas of the likelihood function in parallel are exchanged periodically.
This presentation illustrates an estimation of parameter probability distributions of a three-state gating model depending on a ligand to demonstrate the potential of the developing method.

Keywords: ion channel, gating mechanisms, mathematical modeling, parameter uncertainty, statistical analysis

P13-9

Investigation of drug repurposing strategies according to stages of nonalcoholic fatty liver

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Non-alcoholic fatty liver disease (NAFLD) is a prevalent condition characterized by the accumulation of fat in the liver in individuals who do not consume significant amounts of alcohol. As liver fibrosis progresses, NAFLD can lead to hepatitis, cirrhosis, and liver cancer, which can have a devastating impact on the patient's health and lead to various chronic diseases. With no FDA-approved drugs, drug repurposing has been gaining attention for rapid drug development. Here, we performed Connectivity Map (CMap)-based drug repurposing on transcriptomic data of liver tissues from NAFLD patients categorized into three stages according to steatosis and fibrosis in a previous cohort study. We identified a total of 39 drugs that could reverse the disease gene signature of NAFLD. Eight drugs were common to all three stages, but 17 were found only in the steatosis stage, suggesting fewer treatment options as the disease progresses. The identified drugs were categorized into 29 mechanism of action (MoAs), of which 17 MoAs have already been reported to be associated with NAFLD. Our results may provide insights into the complex association of heterogenous molecular mechanisms involved in NAFLD and possible novel therapeutics.

Keywords: system pharmacology, connectivity map, fatty liver

P14-1

LncRNA-CIR6 induces MSCs to differentiate into cardiomyocytes and prevents myocardial infarction

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Background: The main problem of AMI is that the heart doesn't have the ability to regenerate cardiomyocytes to repair damaged tissue after

infarction, resulting in the heart failing to contract. We found LncRNA-CIR6 can induce non-cardiomyocytes differentiate into cardiomyocytes in our previous study. However, the effect of LncRNA-CIR6 on MSCs is unknown. This study is to observe the induction effect of LncRNA-CIR6 on MSCs in vitro and vivo.
Methods: LncRNA-CIR6 was transfected into BMSCs and hUCMSCs by jetPRIME. After 10 days of transfection, cTnT expression was observed by IHC and FACS. LncRNA-CIR6 was transfected into C57BL/6 mice heart by 100mL of AAV9-cTnT-LncRNA-CIR6-ZsGreen i.v. After 3 weeks transfection followed by AMI surgery, hUCMSCs (5×10^5/100mL)were injected by i.v 1 week later. The heart function was detected by vevo2100 and infarcted size were measured by IHC after 1 week of cell injection. Bioinformatics analysis of LncRNA-CIR6 was performed by AnnoLnc2 database.
Results: LncRNA-CIR6 induce a high percentage of the BMSCs (71.50±0.5774) % and hUCMSCs (95.43±2.130) % to differentiate into cardiogenic cells in vitro as determined by the expression of cTnT. LVEF, LVFE of the AMI heart treated by LncRNA-CIR6 or combined with hUCMSCs injection were significantly increased as well as LVIDd, LVIDs were decreased compared with AMI heart nontreated with LncRNA-CIR6. Compared with AMI group (49.20±1.443%), MI size was more lower after treated by LncRNA-CIR6 (38.40±1.270)% or combined with hUCMSCs injection(24.10±1.386%). The transcriptional expression region of LncRNA-CIR6 was in the Chr17 from 80209290 to 80209536. The predicted secondary and MFE structure showed that the functional region of LncRNA-CIR6 was located at nucleotides 0-50/190-255 in the sequence. LncRNA-CIR6 is predicted to interact with 28 proteins. Among them, only CDK1 is related to cardiomyocyte proliferation and differentiation, while all binding region of CDK1(from 0 to 17) is in the functional region of LncRNA-CIR6 secondary structure.
Conclusion: LncRNA-CIR6 induce BMSCs and hUCMSCs to differentiate into cardiomyocytes in vitro. LncRNA-CIR6 improve heart function and reduce infarcted size probably by inducing hUCMSCs to differentiate into cardiomyocytes in vivo. CDK1 may be involved in the mechanism of inducing effect of LncRNA-CIR6.

Keywords: long non-coding RNA-CIR6, mesenchymal stem cells, cell differentiation, myocardial infarction, cardiac repair

P14-2

CCR5 exhibits distinct roles in bone marrow stromal cells and hematopoietic cells to modulate hematopoietic reprogramming and immunosuppressive activity

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The chemokine system plays a crucial role in regulating leukocyte trafficking during immune and inflammatory responses, while also controlling the survival and effector functions of immune cells. Additionally, it governs important physiological processes, including hematopoiesis and angiogenesis. Of note, the chemokine receptor CCR5 is a critical player in the inflammatory response by guiding leukocytes to sites of inflammation and regulating their activation. However, despite its significance, the precise role of CCR5 in hematopoiesis remains incompletely understood.
In this study, we utilized *Ccr5* knockout mice to investigate the role of CCR5 in the hematopoietic system. Our findings revealed that complete loss of *Ccr5* impairs the function of hematopoietic stem and progenitor cells (HSPCs) by reprogramming cellular energy metabolism, and leads to the generation of immature myeloid-derived suppressor cells (MDSCs).
To determine whether *Ccr5* deficiency has a cell-autonomous role in promoting MDSC production, we conducted bone marrow (BM) transplantation. The $Ccr5^{-/-}$ BM cells were transplanted into lethally irradiated wild-type (WT) mice. Interestingly, we did not observe a significant change in MDSC production between recipients with WT and $Ccr5^{-/-}$ cells. However, when we transplanted WT BM cells into lethally irradiated $Ccr5^{-/-}$ mice, we observed a significant increase in MDSC production. These results suggest that deficiency of BM stromal *Ccr5*, not hematopoietic *Ccr5*, plays a role in modulating HSPC function to produce MDSCs. Moreover, we demonstrated that CCR5 plays a role in modulating the immunosuppressive activity of MDSCs. Taken together, our findings highlight the distinct roles of CCR5 in BM stromal cells and hematopoietic cells in inducing hematopoietic reprogramming for MDSC generation, as well as modulating MDSC immunosuppressive activity. Notably, our study provides new insights into the potential of targeting the CCR5-related pathway in immune response and disease progression.

Keywords: CCR5, hematopoietic stem and progenitor cell, myeloid-derived suppressor cell, bone marrow microenvironment, immunosuppressive function

P14-3

Role of zinc in rat adipose tissue-derived mesenchymal stem cell differentiation

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Background: Adipose Tissue-derived mesenchymal stem cells (AT-MSCs) are able to differentiate into osteoblasts and adipocytes, which play an important role in adipose tissue regeneration. Zinc (Zn) is an essential trace mineral involved in a variety of physiological responses, such as a structural, catalytic, and intracellular as well as intercellular signaling component. However, the effects of Zn on adipogenesis remain unclear. In the present study, we investigated the effect of Zn on adipogenic differentiation in AT-MSCs and its underlying molecular mechanisms.
Methods: The adipogenic differentiation medium supplemented with Zn was used to induce the differentiation of rat AT-MSCs into adipocytes for 12 days. Lipid droplet accumulation was examined by oil red o staining and the mRNA levels of adipogenic markers were evaluated by q-PCR.
Results: Zn significantly increased lipid droplet accumulation and increased the mRNA levels of adipogenic differentiation markers, PPARγ and FABP4, in the rat AT-MSCs.
Conclusion: Our findings suggest that Zn might promote adipogenic differentiation in AT-MSCs.

Keywords: zinc, adipose tissue-derived mesenchymal stem cells, adipogenic differentiation

Poster

Poster

P14-4

Effects of dextromethorphan on the osteogenic and adipogenic differentiation of rat bone marrow-derived mesenchymal stem cells in vitro

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Background: Bone marrow-derived mesenchymal stem cells (BM-MSCs) are able to differentiate into adipocytes and osteoblasts, which play an important role in maintaining normal bone stability. Dextromethorphan (DXM) is a common antitussive drug, but might be harmful for bone such as causing osteoporosis. In present study investigated the effect of DXM on adipogenic and osteogenic differentiation in BM-MSCs and its underlying molecular mechanisms.
Methods: The adipogenic and osteogenic differentiation media supplemented with DXM were used to induce the differentiation of rat BM-MSCs into adipocyte and osteoblast for 12 and 22 days, respectively.
Results: DXM significantly increased lipid droplet accumulation and increased the mRNA levels of adipogenic differentiation markers, PPARγ and FABP4, in rat the BM-MSCs. In contrast, DXM decreased mineralized nodule formation and reduced the mRNA levels of osteogenic differentiation markers, RUNX2 and OCN, in the rat BM-MSCs.
Conclusion: Our data suggest that DXM might promote preferential differentiation of the rat BM-MSCs into adipogenic lineage, but suppress osteogenic differentiation.

Keywords: dextromethorphan, bone marrow-derived mesenchymal stem cells, adipogenic differentiation, osteogenic differentiation

P14-5

Palmitic acid methyl ester promotes cell migration in human bone marrow-derived mesenchymal stem cells

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Bone marrow-derived mesenchymal cells (BM-MSCs) are multipotent stromal cells that play critical roles in regenerative medicine. MSCs can migrate into the injury site, enhance immunomodulation, and improve tissue regeneration. Recent evidence indicated that adipocytes could secrete palmitic acid methyl ester (PAME), a potent vasodilator that can inhibit BM-MSCs proliferation. However, the effects of PAME on BM-MSCs migration remain unclear. To investigate the effects of the PAME on the BM-MSCs migration, the human BM-MSCs were incubated with various concentrations of PAME. The cell *migration* capability after PAME treatment was detected by *wound healing assay. The MTT assay subsequently measured cell proliferation*. The results showed that 30, 50, and 100 μM PAME enhance cell migration to untreated cells after 12 hours. However, detected no significant difference in cell proliferation after treatment. In addition, AA cotreatment with bovine serum albumin, Zn^{2+}, and La^{3+} can significantly inhibit the enhancement of PAME-induced cell migration of PAME for 12 hours. If the structural analog palmitic acid (PA) of PAME is treated, it does not promote migration. Interestingly, SAME can also promote cell migration. According to the experimental results, PAME promotes MSC migration, and stearic acid methyl ester (SAME), which also belongs to methyl ester, also has the same effect. Whether the role of promoting BM-MSCs migration is related to the structure of methyl ester needs further research to be confirmed.

Keywords: palmitic acid methyl ester, bone marrow-derived mesenchymal cells, migration

P14-6

Aldo-keto reductase family 1 member A1 regulates the adipo-osteogenic lineage differentiation by adjusting energy metabolism of mesenchymal stem cells

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Aldo-keto reductase family 1 member A1 (Akr1A1) is a glycolytic enzyme catalyzing the reduction of aldehyde to alcohol. Our study using mesenchymal stem cells (MSCs) from human bone marrow (BMSCs) as well as Wharton's Jelly (WJCs) in combination with gain- and loss-of-function analysis and supplementation of Akr1A1 inhibitor, N6022 demonstrated that Akr1A1 is a master regulator in favor of adipogenesis but detrimental to osteogenesis. More importantly, we are able to promote BMSC differentiation toward osteoblast formation but to block adipogenesis by using N6022. In addition, increased Akr1A1expression was found to inhibit the SIRT1-dependent pathway for modulating gene regulation of transcriptional factors, including PGC-1α (decreased), TAZ (decreased), as well as peroxisome-proliferator-activated receptor γ (PPARγ) (increased) in adipocyte-committed MSCs. Furthermore, the inhibition of the SIRT1-mediated signaling pathway was identified to promote the use of metabolic energy from glycolysis in adipocyte-differentiated cells. In contrast, reduced Akr1A1 expression in osteoblast-committed cells caused to relieve the inhibition of SIRT1-mediated activation for turning on the downstream targets of PGC-1α and TAZ, and finally it facilitates osteogenesis and switches on mitochondrial oxidative phosphorylation for energy production. Our study here suggests that Akr1A1 may play an essential role in the control of adipo-osteogenic lineage fate decision by the SIRT 1-mediating pathway in MSCs.

Keywords: aldo-keto reductase family 1 member A1 (Akr1A1), mesenchymal stem cells (MSCs), peroxisome-proliferator-activated receptor γ coactivator-1α (PGC-1α), transcriptional coactivator with PDZ-binding motif (TAZ), silent mating type information regulation 2 homolog 1 (SIRT1)

P14-7

Development of Bi-functional 3D fibrous scaffold with controlled drug release for osteogenesis-coupled angiogenesis

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We present a novel and facile synthesis of cotton-like three-dimensional (3D) fibrous scaffold containing spatiotemporally defined patterns of simvastatin (SIM) optimized for angiogenesis-coupled osteogenesis. The 3D fibrous scaffolds were functionalized with hydroxyapatite nanoparticles (HA-NPs) to induce the biomineralization process mimicking the apatite-like layer. A 3D fibrous scaffold was fabricated with distinct SIM concentrations and release patterns ranging from days to weeks. The morphology, physiochemical properties, biomimetic mineralization, and drug release of as-fabricated 3D fibrous scaffolds of simvastatin-loaded poly (É›-caprolactone) poly (glycerol-sebacate) hydroxyapatite nanoparticles (3D-PGHS) were investigated. The effects of simvastatin-loaded 3D-PGHS on the osteogenic differentiation of human mesenchymal stem cells (hMSCs) and angiogenesis in human umbilical vein endothelial cells (HUVECs) were investigated. The results show that the 3D-PGHS not only enhanced the expression of osteogenic markers including ALP, RUNX2, and COLA1 in hMSCs but also promoted the migration and tube formation of HUVECs. Our results reveal the potential of 3D scaffold-loaded SIM as a putative point-of-care therapy for tightly controlled tissue regeneration.

Keywords: 3D scaffold, drug delivery, angiogenesis, osteogenesis

P14-8

Tacrolimus improves mesenchymal stem cell function in diabetes by inhibiting DRP1-mediated mitochondrial fission

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Background: Diabetic retinopathy (DR) is a leading cause of blindness in diabetic patients. Human umbilical cord blood-derived mesenchymal stem cells (UCB-MSCs) are emerging as a promising new drug for degenerative disease associated with diabetes. Recent studies have shown that high glucose-increased excessive calcium levels are a major risk factor for mitochondrial reactive oxygen species (mtROS) accumulation and apoptosis. This study aimed to investigate the role of high glucose-induced NFATC1 signaling in mitochondrial oxidative stress-stimulated apoptosis.
Methods: The UCB-MSCs were pretreated with the drugs prior to high glucose treatment. Then, we conducted experiments using western blot, LDH release, mitoSOX staining, qPCR analysis and MitoTacker staining.
Results: High glucose increased cytotoxicity, mtROS, and cleaved caspase-9 expression in UCB-MSCs, and high glucose-induced mtROS was critical for apoptosis. High glucose conditions increased O-GlcNAcylated protein expression and nuclear translocation of NFATC1. However, nuclear translocation of NFATC1 was reduced by ST045849 pre-treatment, an O-GlcNAc transferase inhibitor. Tacrolimus pretreatment recovered high glucose-induced mtROS levels and apoptosis. In DR rat model, subconjunctival transplantation of tacrolimus-pretreated MSCs improved retinal vessel formation, retinal function, and uveitis. In high glucose conditions, tacrolimus pretreatment reduced protein and mRNA expression levels of DRP1 and inhibited mitochondrial fission. In addition, Mdivi-1 pretreatment, DRP1 inhibitor, reduced mtROS levels and apoptosis of UCB-MSCs under high glucose conditions.
Conclusion: In conclusion, we demonstrated that high glucose-induced O-GlcNAcylation activates NFATC1 signaling, important for DRP1-mediated mitochondrial fission and mitochondrial apoptosis.

Keywords: UCB-MSC, mitochondrial dynamics, NFATC1, O-GlcNAc, tacrolimus

P14-9

Amniotic fluid stem cell factors for dry eye symptom relief in a mouse photokeratitis model

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Amniotic fluid stem cells (AFSc) are primitive cells functionally akin to embryonic stem cells, as both can differentiate into multiple cell lineages. However, the AFSc are widely acceptable in both ethical and resourceful aspects. Besides, ASCs are regarded as highly potential in clinical applications due to their promoting effects on cellular proliferation and differentiation. This study investigated the efficacy of dry eye symptom mitigation factors derived from AFSc on an UVB-induced photokeratitis mouse model. The mice were divided into 6 groups (n = 6 in each group): (1) Blank: treated with 0.9% NaCl eye drops only; (2) UVB Damage: UVB exposure, with 0.9% NaCl eye drops; (3) AFSc factors at 1/5 dose: UVB exposure, with 1/5 dose of AFSc factors; (4) AFSc factors at 1/10 dose: UVB exposure, with 1/10 dose of AFSc factors;(5) AFSc factors at 1/20 dose: UVB exposure, with 1/20 dose of AFSc factors; (6) AFT: UVB exposure, with artificial tears. The experiment schedule lasted for 11 days in total. The eye drops, with or without AFSc factors, were administered from Day -2 to Day 8. The UVB irradiation (0.72 J/cm^2) was performed daily from Day 1 to Day 7. Tear volume (TV) and Tear film breakup time (TBUT) were assessed on Day 0, 4, and 7, and ocular surface staining was performed on Day 8, followed by mouse sacrifice for further histopathological analyses. The results showed that AFSc factors could promote TV and TBUT and reduce the ocular surface damage area. Histopathological analyses showed that AFSc factors at 1/10 dose enhanced limbal stem cell mobilization as indicated by increased p63 and PCNA markers, which outperformed the use of artificial tears. In conclusion, AFSc factors could mitigate photokeratitis symptoms comparable to those observed under a dry eye status and may be developed for therapeutic use to relieve dry eye symptoms.

Keywords: amniotic fluid stem cells, tear volume, tear film breakup time, limbal stem cells, dry eye symptoms

Poster

Poster

P14-10

Characteristic of mesenchymal stem cells (MSCs) from Wharton's jelly umbilical cords across different ages of women

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Wharton's jelly of umbilical cord has been proven as a great source of progenitor, stem cells and epithelial cells that have been shown to differentiate into various types, including adipogenic, osteogenic, myogenic, hepatogenic, cardiac, pancreatic, endothelial, pulmonary and neurogenic lineages, in other side of their medical waste shape. Due to their easy isolation, ability to resist immune rejection and ability to differentiate into different types of adult cells, stem cells from umbilical cords are considered superior to other stem cells. Wharton's jelly-derived Mesenchymal Stem Cells (WJ-MSCs) are being continually developed into promising cell therapy. Mesenchymal stem cells (MSCs) are characterized by the expression of CD90+, and CD105+ cell markers, and the absence of CD34 cell markers. CD90+ is a glycoprotein present in the MSCs membranes and also in adult cells and cancer stem cells, while CD105 is a cell surface antigen widely expressed on syncytiotrophoblast and some tissue macrophages. However, since the ageing process has a great impact in senescence of MSCs from bone marrow, so Warthon's jelly derived MSCs are also thought to experience functional decline with systemic ageing, resulting in reduced proliferation, increased senescence, and lower differentiation potential. The possible effect of maternal donor age on the properties of placental and Wharton's jelly derived MSCs has not been thoroughly studied. Here we study the different expression of CD 90+, CD105+ and CD34- stem cells with immunochemistry in Wharton's jelly, based on their reproductive ages, from young mothers (20-30 years old) compared with those from median age mothers (30-35 years old) and old mothers (more than 35 years old), in order to find the efficiency and efficacy of stem cell isolation and culture for cell therapy harvested from human placenta and Warthon's jelly sources. Data was statistically analysed by GraphPad prism. Results shown the average number of CD90+ cells, CD105+ cells and CD34- cells increased with increasing number of MSC with increasing ages although they were not statistically significant (p-value=0.8). These results suggest MSC from different ages of maternal donors do not exhibit significant different.

Keywords: mesenchymal stem cell, stem cell, CD90+/CD105+/CD34-, Wharton's jelly, aging

P14-11

Estrogen deficiency drives TGF-β2-mediated ferroptosis in salivary gland epithelium

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Xerostomia, also known as dry mouth, is a salivary gland (SG) dysfunction characterized by reduced saliva secretion or changes in saliva composition. The prevalence of xerostomia is particularly high among elderly women, indicating a correlation between SG function and estrogen. To investigate the pathogenesis of menopause-related xerostomia, we investigated changes in SG function using ovariectomized (OVX) mice. After surgery, we observed lower estrogen levels, reduced salivation, dilated ducts, and reduced acinar rates. Microarray analysis revealed that genes related to the TGF-β signaling pathway, particularly TGF-β2, and ferroptosis drivers were increased upon OVX. To assess the impact of TGF-β2, we treated the SG-derived organoids (SGOs) with TGF-β2. Interestingly, a dose-dependent decrease in organoid number and proliferation was observed. Furthermore, TGF-β2 treatment increased ferroptosis marker Tfr1 expression and lipid peroxidation, while co-treatment with SB431542, a TGF-β signaling inhibitor, prevented this phenomenon. Finally, treatment of ferroptosis inhibitor liproxstatin restored saliva secretion and reduced ferroptosis marker expression in OVX mice. These findings suggest that TGF-β2 induces SG dysfunction by promoting ferroptosis-induced oxidative stress and cell death.

Keywords: xerostomia, salivary gland, organoid, transforming growth factor-beta 2, ferroptosis

P14-12

Investigating cellular dynamics of live organoids via label-free 3D high-resolution analysis with low-coherence holotomography

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Three-dimensional (3D) visualization is essential for understanding the physiological functions and cell type diversity of live organoids. But conventional 3D imaging techniques based on fluorescence markers have limitations when it comes to long-term, non-invasive observation of live organoids. Holotomography takes advantage of a non-invasive imaging technique since it exploits the refractive index (RI) of a sample as an intrinsic imaging contrast. The RI information can also be translated into quantitative analyses of biomolecular concentration or volumetric analysis of subcellular organelles.
In this study, we employed a low-coherence holotomography imaging system, which is suitable for observing multicellular specimens, to examine the morphological features of murine small intestine organoids (sIOs). With the low-coherence holotomography system, we were able to monitor undisguised live organoids just as they are. We acquired 3D

RI tomograms of live organoids embedded in Matrigel for 120 hours and revealed the early differentiation of sIOs, including the formation of a central cyst structure and crypt-like budding structures. The differentiation of enterocytes, goblet cells, and Paneth cells was distinctly identified through the marker-free observation of subcellular structures such as secretory vesicles and granular structures. Furthermore, we observed cellular dynamics, such as mitotic cell division and the translocation and chromatin condensation of apoptotic cells. Also, we explored the drug response of sIOs by the Cisplatin treatment. We were able to track the shrinkage of crypt due to the cell death, and translocation of a single apoptotic cell.
In conclusion, low-coherence holotomography provides unique capabilities for determining the status of development, differentiation, and viability of organoids, making it a valuable tool for basic research and therapeutic applications. By enabling non-invasive, label-free, and long-term observation of live organoids, this technology has the potential to revolutionize the field of organoid research.

Keywords: 3D organoid imaging, label-free organoid imaging, organoid-drug treatment monitoring, organoid quantification, holotomography

P14-13

Differentiation of expandable endothelial cells from gene-corrected induced pluripotent stem cells for hemophilia a cell therapy

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Background: Hemophilia A is an inherited bleeding disorder caused by various mutations in the coagulation *factor VII* (*FVIII*) gene on chromosome X. Currently, the most commonly used treatment for hemophilia A is the injection of recombinant FVIII, which is inconvenient and expensive for patients. Recently, there has been increasing interest in developing cell therapies based on pluripotent stem cells to develop long-term, effective treatments for hemophilia. Induced pluripotent stem cells (iPSCs), derived from adult somatic cells by transduction of defined reprogramming transcription factors, can be efficiently differentiated into any cell type. Due to their self-renewal and pluripotency, iPSCs have great potential in regenerative medicine. For stem cell-based cell therapies in hemophilia, the amount of FVIII secreted by the transplanted cells has an impact on the therapeutic effect. Large number of ECs and additional expression of FVIII in ECs can be the ways to overcome this problem. Therefore, the aim of this study is to propose a method to obtain a substantial number of homogeneous ECs from iPSCs with enhanced FVIII expression.
Methods: In this study, to enhanced the FVIII expression, we inserted the B-domain deleted form of the FVIII gene in *AAVS1* site of patient-derived iPSCs. We induced mesodermal and vascular differentiation from iPSCs by modulating TGF-β signaling and the SMAD pathway. To obtain a homogeneous population of ECs, magnetic-activated cell sorting (MACS) was performed after vascular differentiation. We measured the proliferative capacity of naïve and cryopreserved ECs and analyzed their characteristics by quantitative polymerase chain reaction (PCR) and immunocytochemistry.
Results: We obtained a homogeneous population of iPSC-derived ECs by MACS. The qPCR results showed that the sorted ECs expressed not only several EC markers such as CD31, vWF and VE-Cad but also FVIII stably. In addition, the ECs maintained their proliferative capacity and characteristics through successive passages. By comparing with non-cryopreserved, we demonstrated that the ECs maintained their proliferative capacity and EC characteristics even after cryopreservation.
Conclusion: In this study, we have shown that a scalable ECs derived from gene-edited iPSCs maintained EC characteristics through passages and had a stable expression of FVIII. Therefore, based on these results, the differentiated ECs we obtained can be considered as potential candidates for cell-based therapy of hemophilia A.

Keywords: iPSCs, hemophilia A, endothelial cells, cell therapy, gene correction

P14-14

The role of FASN-dependent lipogenesis and mitochondria using human derived- iPSCs and cerebral organoids during neurodevelopment

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Lipid synthesis is an important metabolic process that constructs membranes and tissues during the development of nervous system. Lipid synthesis produces various products including fatty acids, phospholipids, glycolipids, sterols, and sphingolipids. Among the various pathways for lipid synthesis, de novo lipogenesis is an endogenous pathway that produces long-chain fatty acids, using the fatty acid synthase enzyme (FASN). In a recent study, human FASN gene point mutation R1819W has been reported with intellectual disability and developmental epileptic encephalopathy. It indicates an association of FASN-dependent lipogenesis metabolism in human brain development and disease. Mitochondria are also important to regulate cell proliferation, differentiation, and maturation precisely and sequentially through mitochondrial dynamics and metabolic shifts during embryonic and neurogenesis in stem cell biology. In a recent study, it is confirmed that mitochondrial dysfunction and apoptosis are induced by inhibition of FASN in iPSCs. This indicates that mitochondria and de novo lipogenesis are essential for maintaining pluripotency and cellular activity of stem cells. Based on discovery that mitochondrial and lipid metabolism suggests the importance of stem cell function and developmental potential. However, it remains unclear how lipid and mitochondrial metabolism are related to neuronal and brain development. To link between FASN-dependent lipogenesis and mitochondria, we mainly focused on between mitochondria and lipid changes on neuronal and brain development in FASN-deficient conditions. Here, we aim to examine: (1) how does deficient-FASN affect neuronal cell maturation and brain development in cerebral organoids (2) how does deficient-FASN affect mitochondria and lipids in human neural stem/progenitor cells (NSPC). Using pharmacological inhibition of FASN in human cerebral organoids, we show that loss of FASN reduced NSPC proliferation, altered neuronal differentiation and affected cellular connectivity. In addition, we use iPSC-derived NSPCs to investigate detailed changes using siRNA system and show that inhibition of FASN altered mitochondrial function and dynamics and affected the cellular lipids and ultrastructure. Thus, our data suggest that de novo lipogenesis and mitochondria are important metabolic processes for the proper development of the human brain. Furthermore, it will help understand energy metabolism related neuronal disorders during brain development.

Keywords: fatty acid, FASN, iPSC, Organoid, neurodevelopment

Poster

P15-1

Integrative single-cell transcriptome analysis reveals new insights into post-COVID-19 pulmonary fibrosis and potential therapeutic targets

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The global COVID-19 pandemic caused by the SARS-CoV-2 virus has resulted in a significant number of patients experiencing persistent symptoms, including post-COVID pulmonary fibrosis (PCPF). This study aimed to identify novel therapeutic targets for PCPF using single-cell RNA-Sequencing data from lung tissues of COVID-19 patients, idiopathic pulmonary fibrosis (IPF) patients, and a rat TGF-β1-induced fibrosis model treated with anti-fibrotic drugs. Patients with COVID-19 had lower alveolar macrophage counts than healthy controls, whereas patients with COVID-19 and IPF presented with elevated monocyte-derived macrophage counts. A differential gene expression analysis showed that macrophages play a crucial role in IPF and COVID-19 development and progression, and fibrosis- and inflammation-associated genes were upregulated in both conditions. Pathway analysis revealed upregulation of inflammation and proteolysis and downregulation of ribosome biogenesis and respiratory gas exchange. Cholesterol efflux and glycolysis were augmented in both macrophage types. The study suggests that antifibrotic drugs may reverse critical lung fibrosis mediators in COVID-19. The results help clarify the molecular mechanisms underlying pulmonary fibrosis in patients with severe COVID-19 and IPF and highlight the potential efficacy of antifibrotic drugs in COVID-19 therapy. Thus, the study's results may have significant implications for the development of new treatment strategies for PCPF.

Keywords: post COVID-19 pulmonary fibrosis, idiopathic pulmonary fibrosis, singe-cell transcriptomics, antifibrotic agent

P15-2

Expression of tyrosine hydroxylase in CD4+ T cells contributes to alleviation of TH17/TREG imbalance in collagen-induced arthritis

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Background: Tyrosine hydroxylase (TH), a rate-limiting enzyme for the synthesis of catecholamines, is expressed in T lymphocytes. Herein, we aimed to show the contribution of TH expression by CD4+ T cells to alleviation of helper T (Th)17/regulatory T (Treg) imbalance in collagen-induced arthritis (CIA), a mouse model of rheumatoid arthritis (RA).
Methods: CIA was prepared by intradermal injection of collagen type II (CII) at tail base of DBA1/J mice. Expression of TH in the spleen and the ankle joints was measured by real-time polymerase chain reaction and Western blot analysis. Percentages of TH-expressing Th17 and Treg cells in splenic CD4+ T cells were determined by flow cytometry. Overexpression and knockdown of TH gene in CD4+ T cells were taken to evaluate effects of TH on Th17 and Treg cells in CIA.
Results: TH expression was upregulated in both the inflamed tissues and the CD4+ T cells of CIA mice. In splenic CD4+ T cells, the cells expressing TH were increased during CIA, and mainly were Th17 cells. TH gene overexpression in CD4+ T cells from CIA mice reduced Th17 cell percentage as well as Th17-related transcription factor and cytokine expression and secretion, whereas TH gene knockdown enhanced the Th17 cell activity. In contrast, TH gene overexpression and knockdown increased and decreased Treg cell activity, respectively.
Conclusion: These findings show that CIA induces TH expression in CD4+ T cells, particularly in Th17 cells, and suggest that the increased TH expression during CIA represents an anti-inflammatory mechanism.

Keywords: tyrosine hydroxylase, CD4+ T cells, helper T 17, regulatory T, collagen-induced arthritis

P15-3

Epithelial hyperactivation of myosin light chain kinase causes bacterial internalization and microbiota dysbiosis to evoke circadian dysrhythmia and chronic inflammation

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Gut barrier defects and microbiota dysbiosis are involved in the pathogenesis of inflammatory bowel diseases (IBD). Circadian dysrhythmia has been reported in IBD patients, which may also play a role in chronic inflammation. Epithelial myosin light chain kinase (MLCK) activation regulated gut barrier functions and caused bacterial endocytosis. Our aim was to investigate whether MLCK-dependent barrier loss promoted microbiota dysbiosis and triggered circadian disruption in IBD models.
Methods: Specific pathogen-free MLCK-transgenic (Tg) and wild-type (WT) mice in different breeding schemes were compared for microbiota composition and colitis susceptibility. Intraepithelial bacteria isolated from colonocytes were used for sequential passaging in epithelial cell cultures *in vitro* and for orogastric inoculation in mouse models *in vivo*. Lastly, human colonic specimens from healthy subjects and IBD patients were analyzed by quantitative PCR.
Results: The presence of intraepithelial bacteria and microbiota dysbiosis were associated with altered circadian gene expression in the Tg mice compared to WT mice in single-housing conditions. More severe chemical-induced colitis and the emergence of intraepithelial bacteria were noted in WT mice after cohousing with Tg mice. Microarray and qPCR analysis showed altered expression in *Nr1d1*, *Clock*, *Per1* and *Nfil3* in the colonic mucosa of WT mice cohoused with Tg mice, which had intraepithelial bacteria. Hemizygote-bred MLCK-Tg littermates harboring postnatal WT microbes displayed *Escherichia* abundance in fecal and epithelial microbiota at an early age, associated with circadian disruption and higher colitis susceptibility than WT littermates in adulthood. Sequential passaging of mouse-isolated intraepithelial *E. coli* in human epithelial cell cultures potentiated the bacterial invasive ability. Inoculation of invasive *E. coli* but not noninvasive commensals led to increased epithelial levels of *Nr1d1* and *Nfil3* associated with proinflammatory cytokine expression in mouse models. Lastly, increased circadian NR1D1 and NFIL3 expression was positively correlated with bacterial abundance in the colonic mucosal biopsy of IBD patients, which were higher than those of healthy individuals.
Conclusion: Epithelial circadian disruption caused by dysbiotic microbiota and intracellular bacteria was an initiator for IBD pathogenicity.

Keywords: epithelial barrier, microbiome, circadian rhythm, invasive pathobionts, enterocolitis

P15-4

Blue light activates retinal endothelial cells and increases inflammatory cytokines release

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The smartphones are prevalent among global people across age groups, especially in young guys. The average time spend on apps and social media is burgeoning year after year, in parallel with the incidence of a variety of ocular diseases. Some of them may due to the excess exposure of the emitting blue light from the displayer. The retina is one of the tissues with the highest metabolic activity, as well as highest vascular circulation. Previously, we demonstrated the endothelial cells are blue-light-responsive, and vulnerable to blue-light-mediated phototoxicity. We anticipate that blue light might activate retinal endothelial cells and in turn stress on retinal tissues. The bEnd.3 and human retinal endothelial cells (HREC) were exposed to low-illuminance blue light (80, 160 and 240 lkkx), followed by the ELISA to quantify the secretion of pro-inflammatory cytokines. We primarily found the interleukin-6 (IL-6), chemokine (C-X-C motif) ligand 1 and 2 (CXCL1 and CXCL2), monocyte chemoattractant protein-1 (MCP-1/CCL2), and platelet-derived growth factor-AA (PDGF-AA) were induced in both testing sets, either in illuminance-dependent or in time-dependent manners. Also, the IkB and P65 was phosphorylated following a short time exposure to blue light. The induction of cytokines was alleviated upon the pre-incubation with pharmacological inhibitors targeting to NFkB (BAY-11-7082). These data support an augmented retinal inflammation by blue-light-illuminated endothelial cells.

Keywords: blue light, retinal endothelial cells, inflammatory cytokines, NFkB

P15-5

Nano-plastics change lipogenesis and induce adipocytokines in 3T3-L1 adipocytes

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After a century of use, the wastes from plastic product were decomposed stepwise by the physical force, insolation weathering and oxidation. The micro-plastics and nano-plastics are plastic particles with the diameter < 5 mm and 100 nm, respectively. They are a new type of environmental pollutant that distributing in the atmosphere, surface water and soil. The contamination of plastic particles has been recognized in aquatic foods, and drinking water. Also, the risk concerns in human health are arising. But, the toxicological profiles of nano-plastic are insufficient for a comprehensive assessment. In this study, by using 3T3-L1 adipocyte, the hazards of nano-PVC to induce metabolic syndromes has been explored. Following 24 h incubation, the nano-PVC did not reduce cell viability, but enhanced the expression level of monoacylglycerol lipase (MGL) and adipose triglyceride lipase (ATGL). The phosphorylation level of hormone-sensitive lipase (HSL) was augmented by nano-PVC. Furthermore, the triglyceride level was accumulated in 3T3-L1 adipocyte. We also found the interleukin-6 (IL-6), granulocyte colony-stimulating factor (G-CSF), and regulated on activation, normal T cell expressed and secreted (RANTES) were induced. These adipocytokines might induce vascular and metabolic disorders, suggesting the undesirable risks of nano-plastics in human health.

Keywords: nano-plastics, adipocyte, lipogenesis, adipocytokines, metabolic syndromes

P15-6

Low expression of SNAREs in antigen-presenting cells causes immune dysregulation in the elderly after single-strand GU-rich viral RNA infection

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The aging immune system is thought to contribute to the severity and fatality of Covid-19 in the elderly population. However, the underlying mechanisms of immune response changes in SARS-CoV-infected elderly are not fully elucidated. Further research is needed to identify these mechanisms and develop interventions to mitigate Covid-19's impact on this vulnerable population.

We developed an animal model to study the effect of single-strand GU-rich SARS-CoV RNAs (CoV ssRNAs) on the immune system. Injection of CoV ssRNAs induced immune cell infiltration in the lungs and resulted in a 40-50% death rate in older mice (>1 year old), while all young adult mice (8-12 weeks old) survived. We used HIV ssRNA40 as a GU-rich RNA control. Analysis of immune cell changes after infection with different ssRNAs revealed unique effects on *in vivo* immune response. Older mice showed fewer lymphoid cells. CoV ssRNA treatment resulted in leukopenia and lymphopenia, while ssRNA40 increased regulatory T cells in both age groups. Moreover, CoV ssRNAs notably impaired inflammatory cytokine secretions, including IL-10, IL-12p70, and TNF, in older mice, but the mRNA expression of these cytokines in $CD11b^+$ cells was not affected. These contradictory results piqued our interest in identifying the pathophysiological mechanism involved.

Previous studies have demonstrated that endosomal Toll-like receptor 7 and 8 (TLR7/8) are involved in the recognition of single-stranded RNA viruses, such as SARS-CoVs. However, the expression of either TLR7 or the TLR adaptor protein MyD88 was unaffected in $CD11b^+$ cells, isolated from CoV ssRNA-treated young and old mice. Our subsequent investigations focused on uncovering the underlying mechanisms of age-related immune dysfunction, with a particular emphasis on cytokine secretion. SNAREs have been shown to play a crucial role in intracellular trafficking of molecules and membranes. Our findings indicated an age-related impairment of SNARE expression in $CD11b^+$ cells, isolated from CoV ssRNA-treated mice. In summary, our study sheds light on the age-related changes in the immune system following SARS-CoV infection and highlights the potential for developing interventions to reduce the severity and mortality of COVID-19 in the elderly population.

Keywords: COVID-19, age, immunity response, Toll-like receptor, SNARE

Poster

P15-7

Infectious cytomegalovirus in breast milk after delivery of preterm infants before 28 weeks of gestational age were measured by the promyelocytic leukemia assay

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Background: In Japan, about 70% of the women of reproductive age are cytomegalovirus (CMV) seropositive. In CMV seropositive mothers, reactivation of latent CMV occurs in postpartum period and the infectious CMV is excreted in breast milk. Infants born before 28 weeks of gestational age from CMV seropositive mothers do not have enough anti-CMV antibodies, therefore, they sometimes develop postnatal CMV infection via breast milk (pCMV-BM). The amount of CMV in breast milk from CMV seropositive mothers is usually evaluated using polymerase chain reaction (PCR) method, however, the viral load from PCR method contains both latent and infectious CMV. Therefore, weekly changes in the amount of infectious CMV are not revealed enough. We measured infectious CMV in breast milk from CMV seropositive mothers using the Promyelocytic leukemia (PML) assay, a patent of Nippi Inc. (Tokyo, Japan), which detects and quantifies only infectious CMV.
Methods: We measured 19 specimens of the surplus breast milk available from 10 CMV seropositive mothers who have delivered preterm infants before 28 weeks of gestational age, maintaining the breastfeeding of their infants. Squeezed and frozen breast milk samples were thawed at 25°C. In the PML assay, whole milk was centrifuged and separated into three layers; lipid pad, aqueous fraction, and cell fraction. *In vitro*, we mixed each layer with 200,000 detecting cells (SE/15 cells) that stably express green fluorescent protein (GFP)-PML protein. Only in the nuclei of CMV-infected SE/15 cells, a certain visible change of GFP-PML protein distribution is observed. This phenomenon is caused specifically by active CMV and not by inactive CMV or other viruses. We count these "PML positive" cells, the number of which indicates CMV infectivity of the sample. We also measured the amount of CMV in the whole milk samples by PCR method, and compared with the result of each sample by the PML assay.
Results: By the PML assay, only cell fraction samples were PML positive. Postpartum weekly changes in breast milk after delivery is described as follows (number of samples (No.), number of PML positive samples vs. negative samples (No.), mean CMV viral load by PCR method (copies/mL), in order):
Week 1: 1, 0 vs. 1, not detected
Weeks 2: 2, 1 vs. 1, 3.4
Weeks 3: 5, 1 vs. 4, 3.8
Weeks 4: 4, 1 vs. 3, 2.4
Weeks 5: 7, 0 vs. 7, 12
Conclusion: By the PML assay, infectious CMV in breast milk was detected within 1 month after delivery. Results of PCR method was not relative to results of the PML assay. Moreover, the cell fraction in breast milk was supposed to be the main source of pCMV-BM. The PML assay is meaningful in revealing kinetics of pCMV-BM and discussing how to prevent it.

Keywords: CMV, preterm, breast milk, PML assay, PCR

P15-8

Impact of Switching from tobacco smoking to vaping on salivary CRP levels in gingivitis patients: A randomized controlled trial

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Smoking has detrimental effect to periodontal health. In smokers, biomarkers of inflammation are upregulated, as well as CRP. Vaping, a safer alternative to smoking, has grown in popularity. The effect of switching from tobacco smoking to vaping on periodontal tissue and salivary CRP levels is unclear. Some research suggests vaping may cause oral health issues. Electronic devices heat a liquid, usually nicotine, without combustion. This study examined salivary CRP levels in gingivitis patients who switch from smoking to vaping. It compares the CRP levels of smokers who switch to e-cigarettes with a control group of tobacco smokers to see how e-cigarettes affect oral inflammation. This was an RCT randomly assigned 40 gingivitis patients to an e-cigarette group and a traditional cigarette group. Smoking was prohibited two hours before saliva collection. Saliva was passively collected in a vial for 10 minutes after ingestion. CRP levels were measured three months after vaping or smoking. Tobacco smokers showed no significant CRP changes. CRP levels were correlated by 0.673 (p-value = 0.001) in the switch group. Three months of vaping decreased salivary CRP levels in the switch group (t-value = 2.155, p-value = 0.044). CRP levels did not correlate with time or the smoking control group. In conclusion, switching from tobacco smoking to vaping in gingivitis patients significantly lowers salivary CRP. CRP levels were unrelated to time or the control group. These data help us understand how switching to e-cigarettes may affect oral inflammation in gingivitis patients.

Keywords: CRP levels, gingivitis, inflammation, smoking, vaping

P15-9

Gas6 signaling induced AIM production to inhibit NLRP3 inflammasome activation

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Previously, we demonstrated a novel role of AIM in the inhibition of inflammasome activation. Based on these previous findings, we, here, investigated whether Gas6 signaling-induced AIM mediates the inhibition of inflammasome activation in mouse bone-marrow derived macrophages (BMDM). First, we demonstrate that treatment with mouse recombinant Gas6 (rGas6) at 100 ng/ml enhanced AIM expression at gene and protein levels in mouse BMDM. We found that treatment with rGas6 inhibited LPS/adenosine triphosphate (ATP)-induced IL-1β and IL-18 production as well as caspase 1 activation, whereas rGas6 had no effects on the enhanced TNF-α. Silencing AIM by two types of specific siRNAs reversed the reduction of LPS/ATP-induced IL-1β and IL-18 production by rGas6. Furthermore, knockdown of AIM in BMDM attenuated rGas6-induced inhibition of caspase-1 activation and apoptosis-associated speck-like protein containing a caspase activation and recruitment domain (ASC) speck formation. Similarly, silencing LXRα by two types of specific siRNA reversed the reduction of LPS/ATP-induced IL-1β and IL-18 production as well as caspase-1 activation by rGas6. Our in vitro data reveal that AIM mediates Gas6 signaling-induced NLRP3

inflammasome activation in BMDM.

Keywords: Gas6, AIM, Inflammasome, LXRα, BMDM

P15-10

Protective effect of TRESK channel against cellular stress induced by hydrogen peroxide and lipopolysaccharide

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Our previous study demonstrated that increased TRESK channels aid motor and sensory recovery after spinal cord injury, which elicits complex pathological responses triggered by inflammation and oxidative stress. This study was conducted to investigate how the TRESK channel regulates inflammation and oxidative stress in dorsal root ganglion (DRG) neurons and PC12 cells exposed to hydrogen peroxide (H_2O_2) and lipopolysaccharide (LPS). Both H_2O_2 and LPS treatments resulted in elevated expression levels of TRESK channels in DRG neurons and PC12 cells. H_2O_2 exposure led to cell death and upregulated markers associated with cell death, such as caspase3, PARP, caspase1, and gasdermin D. On the other hand, LPS treatment stimulated the production of inflammatory mediators including IL-1β, IL-6, TNF-α, and PGE2. Cells overexpressing TRESK exhibited significantly reduced cell death and production of inflammatory mediators compared to cells transfected with the vector. Moreover, DRGs obtained from mice overexpressing TRESK showed diminished inflammatory and oxidative signals compared to those from wild-type mice. Furthermore, TRESK overexpression effectively attenuated the increase in apoptotic, pyroptotic, and inflammatory signals induced by H_2O_2 and LPS compared to the control (vector-transfected cells). These findings support the involvement of TRESK channels in the regulation of inflammation and oxidative stress by attenuating apoptotic and pyroptotic signals and suppressing NF-κB activation.

Keywords: TRESK, dorsal root ganglion, inflammation, oxidative stress, lipopolysaccharide

P15-11

Anti-rheumatoid arthritis effects of sea hare hydrolysates

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Our previous study demonstrated that sea hare hydrolysate (SHH) induces cell death in the A549 lung cancer cell line by activating macrophage type 1 (M1) and suppressing macrophage type 2 (M2), indicating the potential of SHH as an immunotherapeutic agent for cancer treatment. However, as immunotherapeutic drugs may have side effects such as exacerbating rheumatoid arthritis (RA), it is crucial to investigate the effects of SHH on RA. In this study, we investigated the effects of SHH on RA using a well-established collagen-induced arthritis (CIA) mouse model, comparing SHH treatment with the standard RA drug, methotrexate (MTX), to evaluate its effectiveness in arthritis management. The CIA mice treated with SHH showed reduced inflammatory responses in the paws compared to the vehicle group. Both SHH and MTX groups displayed significantly lower arthritis scores and paw thickness. Moreover, the CIA group exhibited increased levels of anti-collagen type II (CII) antibodies, including total immunoglobulin (Ig)G, IgG1, and IgG2a, which were significantly reduced by SHH or MTX treatment. The serum of CIA mice had elevated concentrations of pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) in their serum compared to the vehicle group. However, SHH or MTX treatment led to a significant reduction in these cytokine levels. Histopathological analysis revealed that CIA mice exhibited arthritic inflammation, synovial hyperplasia, and loss of articular cartilage and bone. Both SHH and MTX treatments significantly mitigated these pathological symptoms in the ankle joint. Micro-CT scans of the hind paws also showed a reduction in articular destruction in the SHH and MTX groups. These findings show that SHH treatment decreases the severity of CIA and exerts anti-inflammatory effects by reducing pro-inflammatory cytokine levels and inhibiting the anti-CII antibody production. Therefore, we suggest that SHH holds promise as an immune-anticancer agent without side effects, such as exacerbation of rheumatoid arthritis.

Keywords: rheumatoid arthritis, sea hare hydrolysate, immunotherapeutic, inflammation

P15-12

Extraocular lacrimal gland inflammation and degeneration caused by long-term dry eye status

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The prevalence of dry eye syndrome has been estimated to be between 14.4-24.4% worldwide, i.e. more than 1 billion people suffer from dry eye disease. The pathogenesis of dry eye has been investigated in many aspects. However, a literature search found that mechanisms for extraocular lacrimal gland (ELG) alterations caused by dry eye syndrome remain elusive. Thus, this study aimed to investigate the differences of ELG pathogenesis between the acute and chronic dry eye phases and their relevance with cornea nerve fiber density on the ocular surface. We divided six-week-old ICR female mice into two groups, an acute group and a chronic group, with both groups sub-divided into either a control group or an injury group. The injury was performed by subjecting the mouse cornea to UVB (ultraviolet B) irradiation: (1) Acute control group: no treatment, for 7 days; (2) Acute injury group: UVB irradiation at 0.72 J/cm^2 for 75 seconds daily for 7 days; (3) Chronic control group: no treatment, for 26 days; (4) Chronic injury group: UVB irradiation at 0.72 J/cm^2 for 75 seconds daily for 26 days. The mice were sacrificed on day 8 (acute group) or day 27 (chronic group) histopathological sections were performed. Measurements of tear volume (TV), tear breakup time tests (TBUT), and ocular surface photography were conducted at different day intervals before the final sacrifice, followed by histopathological analysis for inflammatory (Cox2 and NF-κB) and oxidative stress (8-OHDG and 4-HNE) markers as well as α-SMA protein of myoepithelial cells. The results indicate that acute and chronic injuries lead to decreased tear volume and a shortened tear break-up time. Regarding the ocular surface, the chronic group exhibited a larger damage area than the acute group. Furthermore, we found that both the injury groups exhibited elevated oxidative stress, acinar interstitium enlargement,

and increased deposition of reddish-brown precipitates. However, the acute injury group showed the presence of fibrosis. In short, it can be concluded that acute dry eye syndrome causes more significant damage compared to the chronic group. These findings will lead to more in-depth analyses of extraocular lacrimal gland pathogenesis caused by dry eye.

Keywords: acute and chronic dry eye syndrome, lacrimal gland, glandular inflammation and degeneration, oxidative damage, mechanism

P15-13

Galectin-9-expressing infiltrated macrophages alleviate liver fibrosis by inhibiting activated hepatic stellate cells through Tim-3 signaling

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Background: Liver fibrosis, a healing response to hepatic damages, is defined as an excessive accumulation of extracellular matrix and as a common process in the development of chronic liver diseases, it is important to find a solution for the treatment. Recently, a correlation between serum galectin-9 and liver fibrosis has been reported. Galectin-9 is one of the most well-known ligands for the immune checkpoint T-cell immunoglobulin mucin domain containing-3 (Tim-3), and role of their interactions in liver fibrosis remain uncleared. As such, we set out to investigate whether a Gal-9/Tim-3 signaling axis has a regulatory function in liver fibrosis.
Methods: C57BL/6 wild-type (WT) mice, Tim-3 knockout (Tim-3KO) mice, and hepatic stellate cell (HSC)-specific conditional knockout of Tim-3 (Tim-$3^{\Delta HSCs}$) were all subjected to induced liver fibrosis by intraperitoneally injection of CCl_4. The levels of liver fibrosis were evaluated through biochemical and histological analyses of both blood and liver tissues. Additionally, qRT-PCR was utilized to assess the extent of fibrosis in the liver. The level of Galectin-9 and Tim-3 were quantified in liver tissues and liver immune cells using qRT-PCR and flow cytometry analysis, and visualized by immunofluorescent staining. Primary hepatic stellate cells (HSCs) were isolated and cultured *in vitro* for treating with recombinant galectin-9.
Results: Compared to control group, C57BL/6 mice with liver fibrosis showed a significant increase of both mRNA and protein level of galectin-9 in blood and isolated liver immune cells. Among various liver immune cells, $CD11b^+Ly6G^+$ neutrophils and particularly $CD11b^+F4/80^+$ infiltrated macrophages notably elevated galectin-9 expression in qRT-PCR and FACS analysis, and through immunofluorescent staining, these galectin-9^+ immune cells were found to be localized in fibrotic septa. Comparing CCl_4-injected C57BL/6 groups, Tim-3KO groups showed a more severe level of liver fibrosis as indicated by Sirius red staining and mRNA level of *Acta2* and *Col1a1* in whole liver tissues, indicating more activated level of HSCs in Tim-3KO mice. Interestingly, non-immune HSCs increased mRNA level of Tim-3 upon *in vitro* activation, which corresponded with HSCs RNA-sequencing data. Moreover, when HSCs were cultured *in vitro*, mRNA levels of Tim-3 increased significantly upon activation. Furthermore, when HSCs isolated from both C57BL/6 and Tim-3KO were treated with recombinant galectin-9, activated HSCs from C57BL/6 group decreased mRNA level of *Acta2* and *Col1a1* in dose-dependent manner whereas activated HSCs from Tim-3KO did not show changes in mRNA level.
Conclusion: Our data suggests that the galectin-9^+ macrophages play a crucial role in alleviating the liver fibrosis by inhibiting activation of HSCs through Tim-3 signaling. With the abovementioned results, we suggest Gal-9/Tim-3 signaling as a potential therapeutic target of liver fibrosis.

Keywords: liver fibrosis, hepatic stellate cells, immune checkpoints, T-cell immunoglobulin mucin containing domain-3, Galectin-9

P15-14

Evaluation of the anti-inflammatory potential of Broncho-Vaxom® (OM-85 BV) in a mouse model of lipopolysaccharide-induced acute lung injury

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Acute Lung Injury (ALI) is a condition associated with acute respiratory failure, resulting in significant morbidity and mortality. It involves cellular changes such as disruption of alveolar-capillary membrane, excessive neutrophil migration, and release of inflammatory mediators. Broncho-Vaxom® (BV), a lyophilized product containing cell membrane components derived from eight bacteria commonly found in the respiratory tract, is known for its potential in reducing viral and bacterial lung infections. However, the specific impact of BV on ALI remains unclear. This study aimed to investigate the preventive effects of BV and its underlying mechanism in a lipopolysaccharide (LPS)-induced ALI mouse model. BV (40 mg/kg) was administered one hour before intratracheal LPS injection to assess its preventive effect in the ALI model. Pre-administration of BV effectively mitigates inflammatory parameters, including increased secretion of inflammatory mediators, macrophage infiltration, and NF-κB activation in lung tissue, and an increase in inflammatory cells in bronchoalveolar lavage fluid (BALF). In addition, BV (100 μg/mL) pretreatment reduced the expression of M1 markers, interleukins (IL-1β, IL-6), tumor necrosis factor α, and cyclooxygenase-2, which are activated by LPS, in alveolar macrophage MH-S cells. These results showed that BV exhibited anti-inflammatory effects by suppressing inflammatory mediators, suggesting its potential in attenuating bronchial and pulmonary inflammation.

Keywords: acute lung injury, Broncho-Vaxom, inflammation, lipopolysaccharide, macrophage

P15-15

Impact of clonal hematopoiesis on the Pathophysiology of COVID-19 in rhesus macaques

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Despite groundbreaking research and the development of treatments and vaccines, a significant number of patients with COVID-19 still suffer from life-threatening clinical outcomes. Clinical manifestations of COVID-19 vary, ranging from asymptomatic to lethal respiratory failure with multiorgan dysfunction. Although several risk factors for late hyperinflammatory COVID-19 illness have been revealed to date, most

notably age and pre-existing comorbidities, the core factors leading to severe clinical manifestations remain unelucidated. Clonal hematopoiesis (CH), acquired somatic mutations in certain genes such as *DNMT3A* and *TET2* in hematopoietic stem and progenitor cells (HSPCs), is associated with hyperinflammation as well as clonal expansion of mutant HSPCs. Based on the similar age range of frequent CH and severe COVID-19 disease, we hypothesized the presence of CH could impact the severity and pathophysiology of the SARS-CoV-2 infection. A few human cohorts have attempted to explore this relationship, but results to date are somewhat controversial. Rhesus macaques (RMs) have been reported as a good model of SARS-CoV infection for the development of vaccines and therapies, but up to now, no models available that mimic late hyperinflammatory COVID-19 illness. In the previous study, we created a robust RM model of CH through autologous transplantation of HSPCs introduced loss-of-function CH mutations by CRISPR technology. To demonstrate whether the RMs with CH could develop severe late COVID-19 disease and be utilized as a model to study disease pathophysiology or test therapeutic approaches, macaques with either natural CH (n=1) or engineered (n=2) along with non-CH controls receiving the same total body irradiation and transplantation regimen (n=3) were inoculated with SARS-CoV-2 and monitored until 12 days post-inoculation (dpi). An aged animal with natural *DNMT3A* CH died suddenly on 10 dpi, although no remarkable differences in clinical symptoms and blood counts between the two groups. CH animals showed somewhat elevated serum levels of IL-6 until 12 dpi, and in bronchoalveolar lavage (BAL), mean concentrations of MCP-1, IL-6, IL-8 and MIP-1b were consistently higher in CH macaques compared to controls. Interestingly, we found the median copy number of subgenomic SARS-CoV-2 RNA was higher at every timepoint in the CH group as compared with the control group, in both upper and lower respiratory tracts. Lung sections from euthanasia at 10 or 12 dpi represented evidence of mild inflammation in all animals. However, while the viral antigen was detected in the lung tissues of all the CH animals even at the time of euthanasia, only one animal of controls had detectable SARS-CoV-2 antigen. Although the marked inflammation and serious illness have not been observed, our results indicate potential pathophysiological differences in RMs with or without CH following SARS-CoV-2 infection.

Keywords: Rhesus macaques, clonal hematopoiesis, COVID-19, SARS-CoV-2, Hyperinflammation

P15-16

The new role of ferroptosis progress in Psoriasis disease

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Background: Psoriasis is a chronic inflammatory skin disease with a strong genetic predisposition and autoimmune pathogenic traits. It characterizes by sharply demarcated, erythematous, pruritic plaques covered in silvery scales. The pathogenesis of psoriasis relates to immune cells and the increase of proinflammatory cytokines, especially in the IL-23/Th17 axis. Recently, there are many studies found the role of ferroptosis in the pathogenesis of many diseases related to the immune system such as acute kidney injury, intracerebral hemorrhage, and neurodegenerative diseases. So, in this study, we investigate the process of ferroptosis and some related genes in the Psoriasis model.
Methods: We induce the Psoriasis model on mice with 5% Imiquimod cream and induce the ferroptosis model on keratinocyte cells by erastin. Investigate some ferroptosis makers and related genes in these models. Skin parameters were measured and investigate skin lesions by hematoxylin and eosin staining. Western blot was used for protein levels evaluation and mRNA levels were measured by RT-PCR.
Results and Discussion: There are proliferation and thickening of epidermal keratinocyte cells besides the increase in the water loss index and a significant decrease in the skin hydration index as well melamine index decreased, and the erythema index increased in the skin hydration index in a psoriasis model. Moreover, there are changes in ferroptosis makers such as GPX4, and ACSL4, and the increase of proinflammatory cytokines in vivo Psoriasis model. We also found that keratinocyte is sensitive to ferroptosis inducer and ferroptosis in this cell line has related to some genes that we investigate.
Conclusion: The study shows the role of the ferroptosis process and some related genes in the Psoriasis model.

Keywords: ferroptosis, psoriasis, cell death program

P15-17

Pharmacokinetics of steppogenin in mouse depend on its route of administration

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We developed and validate a sensitive analytical method to analyze steppogenin in mouse plasma using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The standard calibration curves for steppogenin ranged from 1 ng/ml to 1000 ng/ml, and were linear with a mean CV and accuracy of 8.65% and 101.0%. We used the protein precipitation method using methanol for steppogenin sample preparation and naringenin as an internal standard. The inter- and intraday accuracy and precision as well as stability (i.e., three cycles of freeze-thaw stability, short-term stability, and processed sample stability) in three quality control samples fell within acceptance criteria. Also, we investigate the pharmacokinetics of steppogenin with different route of administration. Steppogenin were administered via intravenous (IV, 0.34 mg/kg), intraperitoneal (IP, 0.3 mg/kg), subcutaneous (SC, 0.3 mg/kg), and per oral (PO, 0.45 mg/kg) route. The absolute bioavailability of steppogenin was different depend on the administration route: 54.6% for IP, 96.0% for SC, and 8.8% for PO.

Keywords: LC-MS/MS, steppogenin, route of administration, pharmacokinetics

P15-18

Bile acids direct neutrophils to maintain intestinal homeostasis through NET formation

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Bile acids (BAs) are generally considered to be potent endocrine hormones that regulate physiological responses such as lipid and bile acids homeostasis. In this study, we found that BAs stimulate neutrophil extracellular traps (NETs) formation through farnesoid X receptor (FXR), bile acids-regulated nuclear receptor. BA-induce NETs provide a barrier in the small intestine against intestinal bacteria. Chenodeoxycholic acid (CDCA), a primary bile acid, significantly enhanced NETs formation and GW4064 and obeticholic acid (OCA), the synthetic agonists for FXR, also enhanced NETs formation, whereas neutrophils isolated from $FXR^{-/-}$ mice did not generate NET formation in response to BAs. Moreover, CDCA enhanced expressions of citrullinated histone 3 (cit-H3) in neutrophils

and inhibitors for protein arginine deaminase 4 (PAD4) attenuated CDCA-induced NETs formation. As the increase of intracellular calcium is required for PAD4 activation, we further examined the effects of BAs on intracellular calcium levels in neutrophils. CDCA and FXR agonists significantly enhanced intracellular calcium levels in both human and mouse neutrophils. We found the NETs in the intestinal crypts of small intestines in wild-type mice which were disappeared in the fasted mice. Interestingly, re-feeding restored NETs in the small intestines. $FXR^{-/-}$ mice did not contain intestinal NETs and showed increased the dissemination of bacteria into distant organs such as liver, mesenteric lymph node, and spleen. The dissemination of orally inoculated *Salmonella* was higher in $FXR^{-/-}$ than wile-type mice. In conclusion, we found that the BAs direct neutrophils to maintain intestinal homeostasis through NETs formation which restricts systemic dissemination of bacteria obtained under nutrient flow.

Keywords: Farnesoid X receptor, chenodeoxycholic acid, neutrophil extracellular trap, intestinal homeostasis

Poster

P15-19

The identification of splenic B-helper neutrophils in a murine model of transplantation

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Neutrophils, the most common type of cell among leukocytes in the human body, play a crucial role in innate immunity. Transplantation refers to the surgical procedure of transferring organs, tissues, or cells from a donor to a recipient. During transplantation, neutrophils contribute to acute and chronic inflammation and act as antigen-presenting cells by delivering antigens through draining lymph nodes. B cells sensitize transplant-derived antigens and produce antibodies against these antigens, resulting in antibody-mediated chronic transplantation rejection. In this study, we examined the role of neutrophils in antibody-mediated chronic transplantation rejection. To investigate this hypothesis, we utilized an allogenic skin-graft for transplantation model. Recipient wild-type C5BL/6 mice were sensitized with skin graft from transgenic HLA-A2 mice (C57BL/6-Tg(HLA-A2.1)1Enge/J, and further transplanted with second skin graft for induction of antibody-mediated graft rejection. We conducted single-cell RNA sequencing (scRNA-seq) on splenic cells isolated from syngenic and allogenic group, and examined neutrophil subsets. We found the specific neutrophil subset which express higher levels of B-cell interacting molecules including B-cell activating factors (BAFFs) and genes involved in neutrophil extracellular trap (NET) formation. These results suggest the existence of specialized neutrophil which might be involved in the antibody-mediated chronic rejection in transplantation.

Keywords: neutrophil, B-cell activating factor, transplantation, single-cell RNA sequencing, antibody-mediated chronic transplantation rejection

P15-20

Neutrophils fuel the development of NAFLD through lipid delivery to hepatocytes from adipose tissue

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Nonalcoholic fatty liver disease (NAFLD) is a chronic liver disease characterized by hepatic fat accumulation without alcohol intake. Innate immune cells are recruited into fatty liver and induce steatohepatitis during the progression of NAFLD. Neutrophils, the most abundant innate immune cells, are also involved in the inflammatory process of NAFLD. In this study, we investigated the role of neutrophils in the pathogenesis of NAFLD and found that neutrophils fuel the development of NAFLD through lipid delivery to hepatocyte. Neutrophils exposed to free fatty acids exhibited increased numbers of lipid droplets and the inhibition of lipid transporters completely inhibited lipid uptake of neutrophils. FFA-exposed neutrophils did not show significant changes in neither apoptotic rates nor chemotaxis. Neutrophils co-cultured with adipocytes also exhibited increased numbers of lipid droplets, suggesting lipid-laden capability of neutrophils. To further examine whether neutrophils deliver lipids to hepatocytes, we examined the lipid accumulation in hepatocytes co-cultured with lipid-laden neutrophils. The hepatocytes cultured with lipid-laden neutrophils exhibited increased numbers of lipid droplets, suggesting the lipid-delivering capability of neutrophils. To confirm the existence of lipid-delivering neutrophils, we evaluated the lipid accumulation in neutrophils in a murine model of NAFLD. Peripheral neutrophils isolated from mice fed with high fat diet (HFD) showed increased numbers of lipid droplet compared to control mice fed with normal chow. Interestingly, liver-infiltrating neutrophils in NAFLD mice also exhibited increased numbers of lipid droplets. Finally, we found the existence of lipid-laden neutrophils in the patients with NAFLD. These findings suggest the possible role of neutrophils during the fat accumulation in the pathogenesis of NAFLD.

Keywords: neutrophils, NAFLD, NASH, lipid uptake, lipid-laden neutrophils

P16-1

Mitochondrial matrix protein LETMD1 maintains thermogenic capacity of brown adipose tissue

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Brown adipose tissue (BAT) has abundant mitochondria with the unique capability of generating heat via uncoupled respiration. Mitochondrial uncoupling protein 1 (UCP1) is activated in BAT during cold stress and dissipates mitochondrial proton motive force generated by the electron transport chain to generate heat. However, other mitochondrial factors required for brown adipocyte respiration and thermogenesis under cold stress are largely unknown. Here, we show LETM1 domain-containing protein 1 (LETMD1) is a BAT-enriched and cold-induced protein required for cold-stimulated respiration and thermogenesis of BAT. Proximity labeling studies reveal that LETMD1 is a mitochondrial matrix protein. Letmd1 knockout male mice display aberrant BAT mitochondria and fail to carry out adaptive thermogenesis under cold stress. Letmd1 knockout BAT is deficient in oxidative phosphorylation (OXPHOS) com-

plex proteins and has impaired mitochondrial respiration. In addition, BAT-specific Letmd1 deficient mice exhibit phenotypes identical to those observed in Letmd1 knockout mice. Collectively, we demonstrate that the BAT-enriched mitochondrial matrix protein LETMD1 plays a tissue-autonomous role that is essential for BAT mitochondrial function and thermogenesis.

Keywords: BAT, mitochondria, thermogenesis, Ucp1, OXPHOS

P16-2

Impaired mitophagy of pulmonary arterial smooth muscle and altered mitochondrial dynamics in monocrotaline-induced pulmonary arterial hypertension model

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Pulmonary arterial hypertension (PAH) is associated with various changes of signaling and metabolic pathways in PA smooth muscle cells (PASMC). We conducted proteomic analysis of PASMCs obtained from normal (CON) and monocrotaline-injected PAH (MCT-PAH) rats. In addition to the alterations in contractile machinery proteins, increase of proteasome/lysosome pathways and decrease of pyruvate/fatty acid metabolism pathways were noted. Electron microscopy of pulmonary arterial tissues also showed increased mitochondrial fission and mitochondria enclosed with autophagosomes in MCT-PAH. Immunoblot study showed upregulated lysosomal-associated membrane protein 2 (LAMP2) and increased LC3-II/LC3-I ratio, p62 and TOMM20, implying increased formation of autophagosome while incomplete degradation of the mitochondria. The expression of mitochondrial uncoupling protein 2 (UCP2) and anti-ATPase inhibitory factor 1 (ATPIF1) were decreased and increased, respectively, in MCT-PAH. Also, AMPK phosphorylation (p-AMPK/AMPK) and acetyl-CoA carboxylase phosphorylation (p-ACC) were decreased in MCT-PAH. Oxidative phosphorylation analysis revealed decreased levels of maximal respiration, spare respiratory capacity, and OCR/ECAR in the PASMCs primarily cultured from MCT-PAH than CON. Taken together, in the MCT-PAH PASMCs, it is suggested that increased lysosome/proteasome pathway and impaired autophagy/mitophagy flux have resulted accumulation of damaged mitochondria, abnormal energy metabolism. The above features might play roles in the pathophysiology and vascular remodeling in PAH.

Keywords: pulmonary arterial hypertension, mitochondria, mitophagy

P16-3

Effects and mechanisms of mitochondrial transplantation in Ang II-induced preeclampsia rats

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Background: Preeclampsia (PE) is a disease mainly caused by placental ischemia, mitochondrial transplantation has been shown to be effective against ischemic diseases. We aimed to study the effect of mitochondrial transplantation in a rat model of PE.
Methods: PE was induced by infusing angiotensin II via mini-osmotic pump (1 μg/kg/min) from day 8 of gestation (GD 8). Mesenchymal stem cell mitochondria from umbilical cord (100 μg/μl) was injected through jugular vein on GD 14. Rats were sacrificed on GD20 and mitochondria were isolated from placenta for molecular analysis.
Results: Blood pressure was increased and glomerulus was enlarged with diminished capillaries in Ang II rats. Fetal weight and fetal crown rump length were significantly decreased and sFLT-1 mRNA in placenta was higher but placental growth factor (PlGF) was decreased, resulted in greater sFLT-1/PlGF ratios in Ang II rats. IHC staining of eNOS indicated significantly smaller vascularization in Ang II placenta. Mitochondria transplantation decreased blood pressure, reversed glomerulus morphological changes, increased fetal weight and crown rump length and decreased sFLT-1 and sFLT-1/PlGF ratio, restored placental vascularization, suggesting establishment of PE with Ang II and therapeutic roles of mitochondrial transplantation in PE rats. Functionally, membrane potential was lower and ROS was elevated in the placental mitochondria from PE rats. Along this line, mitochondria transplantation reduced mitochondrial fission markers (DRP1, FIS 1), which was increased in PE placenta and increased mitochondrial fusion markers (OPA1, MFN1, MFN2).
Conclusion: Ang II induced PE and reduced mitochondrial function in placenta. Mitochondria transplantation restores cardiovascular phenotypes in PE by affecting dynamic mitochondrial functions. We speculate that mitochondrial transplantation is an effective therapy to reduce the cardiovascular damage in PE.

Keywords: mitochondria transplantation, preeclampsia, angiotensin II, sFLT-1

P16-4

Reduced MPST/H2S system and myocardial mitochondrial dysfunction in diabetic rats

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Background: Hydrogen sulfide (H2S) plays protective roles in heart diseases. 3-mercaptypyruvate sulfertransferase (MPST) generates H2S in the mitochondria. Its downregulation in diabetic heart mediates cardiac pathology.
Objective: To explore MPST regulation of mitochondrial function in rat model of type 1 diabetic myocardium.
Method & Results: Blood glucose concentration was elevated post-streptozotocin (STZ) injection in SD rats (55mg/kg ip, 4 weeks). Food/water intake and urine excretion were increased and body weight was decreased. OGTT revealed uncontrolled blood glucose levels, confirming type 1 diabetes. Reactive oxygen species (ROS, amplex red) and membrane potential (TMRM) were decreased in isolated mitochondria from left ventricle, while oxygen consumption capacity was increased in diabetes, indicating mitochondrial dysfunction. mRNA expressions of PGC1-α, CD36 and calreticulin were increased in diabetics and ERR1-α, HMGB or SQOR mRNA expressions were unchanged. Plasma and mitochondrial H2S were increased but protein and mRNA expressions of MPST were reduced in diabetics. In contrast, cystathionine gamma-lyase (CTH), a cytosol H2S generating enzyme, was increased in the heart tissue of diabetes. Further analysis showed that fusion-related proteins, Opa1 was reduced but MFN1/MFN2 were increased in diabetic mitochondria. DRP1 and FIS1, mitochondrial fission-related proteins, were unchanged.
Conclusion: Mitochondrial functions were reduced in type 1 diabetic rat hearts. Mitochondrial MPST was decreased but increased cytosol CTH may compensate for H2S reduction and maintains mitochondrial biogenesis and mitochondrial dynamics. The insights of H2S and its origin and targets in cardiac mitochondria of type 1 diabetes needs fur-

Poster

ther investigation.

Keywords: diabetes, H2S, mitocondria, MPST

P16-5

Roles of mitochondria in energy metabolism after heartbeat initiation in the heart primordium of the rat embryo

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Background: Heartbeat in a rat embryonic heart primordium begins at around embryonic day 10.0 (E10.0) via extracellular calcium influx through L-type calcium channels. However, it remains unclear if and how mitochondria, which play a central role in energy production in eukaryotic cells, are associated with energy metabolism after heartbeat initiation in the rat embryo.
Methods: The heart primordium at E10.0 before and after heartbeat initiation and the primordial heart tube at E11.0 were isolated from Wistar rat embryos, and these samples were subjected to biochemical or physiological experiments including metabolome analysis, gene expression analysis using a DNA microarray, proteomic analysis using data-independent acquisition mass spectrometry, and metabolic flux analysis using an extracellular flux analyzer to identify roles of mitochondria in energy metabolism upon heartbeat initiation in the early rat embryo.
Results: Principal component analysis of the metabolome analysis revealed that the top three determinants in the heart primordium after heartbeat initiation compared with those before heartbeat initiation were ATP, the major product of glucose catabolism; reduced glutathione, a byproduct of the pentose phosphate pathway with antioxidant properties; and GTP, a metabolite that plays roles in protein synthesis and the cytoskeletal system. Genes associated with striated muscle contraction, glycolysis, and calcium handling were up-regulated in the heart primordium after heartbeat initiation compared with those before heartbeat initiation. An extracellular flux analysis showed that ATP-linked respiration and glycolytic capacity were increased in cells from the heart primordium after heartbeat initiation, suggesting that glucose oxidation was enhanced after heartbeat initiation. Finally, although there were no differences in protein expression levels of subunits in mitochondrial oxidative phosphorylation (OXPHOS) in the heart primordium before and after heartbeat initiation, expression levels of proteins that are involved in both the TCA cycle and OXPHOS were increased in the primordial heart tube at E11.0.
Conclusion: The results suggest that glucose oxidation is increased at the initial heartbeat in the heart primordium to support excitation-contraction coupling and that biosynthesis or maturation in mitochondria is enhanced during the period of development from the heart primordium to the primitive heart tube in rats.

Keywords: mitochondria, heartbeat initiation, energy metabolism, development, glucose oxidation

P16-6

C-terminus of caveolin-1 regulates mitochondrial stress response in vascular smooth muscle cells

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Vascular smooth muscle cells (VSMCs) are critical in the repair of vascular injury caused by mechanical stress and inflammation. When the injury occurs, VSMCs rapidly polarize in response to growth factors such as platelet-derived growth factor (PDGF)-BB to induce repair. Mitochondria are rapidly fragmented during polarization and move to the leading edge, where high energy is in demand, to produce ATP. However, the molecular mechanisms are unknown. Caveolin 1 (Cav1) is an integral membrane protein abundant in VSMCs. We reported that Cav1 localizes to mitochondria and plays a non-canonical role in regulating mitochondrial function. Moreover, it was reported that the expression of genes involved in mitochondrial function was abnormal in fibroblasts expressing mutated Cav1, in which the C-terminal 18 amino acid residues are truncated. In this study, we examined the role of Cav1 in mitochondrial stress response in VSMCs, focusing on the C-terminus.
Lentiviral vectors with a truncated C-terminal 18 amino acid residue of human Cav1 cDNA, full-length Cav1, or eGFP-control were transfected into primary human VSMCs (mutant-Cav1-VSMCs, full-Cav1-VSMCs, control-VSMCs). We examined the impact of regulated mitochondrial responses to short-term PDGF-BB stimulation. Mitochondrial respiration was examined using an extracellular flux analyzer. The maximum capacity of the electron respiratory chain reflects the stress response potential. Maximal respiration of mutant-Cav1-VSMCs was significantly decreased compared to full-Cav1-VSMCs and control-VSMCs. PDGF-BB stimulation significantly increased maximal respiration in full-Cav1-VSMCs and control-VSMCs, but not in mutant-Cav1-VSMCs. Morphological changes of mitochondria with PDGF-BB stimulation for 15 min were examined by live cell imaging. Rapid mitochondrial fragmentation was observed in full-Cav1-VSMCs and control-VSMCs, but not in mutant-Cav1-VSMCs. Western Blotting was used to measure the expression of proteins that regulate mitochondrial morphological changes. Expression of mitofusin1 and mitofusin2, which promote mitochondrial outer membrane fusion, was significantly decreased in mutant-Cav1-VSMCs compared to control-VSMCs with or without PDGF-BB addition. Whereas optic atrophy-1, which promotes mitochondrial inner membrane fusion, was not significantly different between mutant-Cav1-VSMCs and control-VSMCs, with or without the addition of PDGF-BB. In addition, phosphorylation of dynamin-related protein 1, which attaches to the mitochondrial outer membrane and promotes mitochondrial fission, was significantly increased in control-VSMCs with PDGF-BB addition for 15 min, but not in mutant-Cav1-VSMCs.
These results suggest that the C-terminus of Cav1 in VSMCs regulates mitochondrial morphological changes from the outer mitochondrial membrane and plays important roles in mitochondrial stress response.

Keywords: caveolin-1, mitochondrial stress response, vascular smooth muscle cells

Poster

P16-7

Etoposide-induced 2.4 (EI24) regulates mitochondrial activity and mitochondrial dynamics

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Background: Mitochondria are essential for various cellular processes, including metabolism, energy production, and signaling. Maintaining the proper functioning of mitochondria is crucial for cell viability, especially under changing conditions. Therefore, mitochondria are dynamic organelles that balance fission and fusion in order to adapt to cellular needs. Interactions between mitochondria and the endoplasmic reticulum (ER) are particularly important for cellular processes, making this contact site extensively studied. A recently discovered ER protein called Etoposide-induced 2.4 (EI24) has been identified as an interacting partner with the outer mitochondrial membrane (OMM). However, the specific role of EI24 in mitochondrial function remains largely unknown. This study aims to investigate the contribution of EI24 to mitochondrial homeostasis, particularly in maintaining mitochondrial dynamics.
Methods: The study focused on hepatocellular carcinoma and multiple invasive cancer cell lines (HepG2, Huh7, MDA-MB 231, and B16F10). Oxygen consumption rate (OCR), mitochondrial membrane potential (MMP), mitochondrial pH, and ATP production were measured in cells with depleted EI24. Mitochondrial dynamics were examined using live-cell confocal imaging under different conditions, including basal, mitochondrial stress, ER stress, hypoxic stress, and viral infection.
Results and Discussion: The results revealed that knockdown of EI24 disrupts the proton concentration in the space between the mitochondrial membranes, leading to a lower mitochondrial membrane potential and increased acidity in the mitochondrial matrix. Consequently, ATP production significantly decreased in EI24-depleted cells. To compensate for the insufficient ATP production, these cells increased their OCR, resulting in elevated levels of reactive oxygen species (ROS) in the mitochondria. Since mitochondrial activity and dynamics are closely linked, the study also explored the effect of EI24 depletion on mitochondrial morphology. Under basal conditions, cells lacking EI24 had elongated mitochondria similar to control cells. However, under various stress conditions, EI24-deficient cells were unable to maintain their elongated mitochondrial structure. Additionally, EI24 knockdown reduced the expression of fusion proteins involved in mitochondrial fusion (mitofusins and OPA1) and increased fission by promoting the phosphorylation of dynamin-related protein 1 (DRP1) at residue S616 during stress conditions.
Conclusion: This study identified a novel function of EI24 in maintaining mitochondrial activity and dynamics. The findings indicate that EI24 is involved in regulating mitochondrial fusion and fission processes. Under normal conditions, EI24-depleted cells exhibited elongated mitochondria similar to control cells. However, when exposed to various stress conditions, cells lacking EI24 were unable to maintain their elongated mitochondrial structure.

Keywords: mitochondria, mitochondrial dynamics, EI24

P16-8

The critical role of TWIK1 in hair cell survival against noise-induced hearing loss

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Noise-induced hearing loss (NIHL) is a condition characterized by auditory impairment resulting from prolonged or intense exposure to loud noises. While previous studies have suggested a potential association between K2P channels and NIHL, the precise mechanism underlying the involvement of TWIK1, a member of the K2P potassium channel family, in NIHL remains elusive. In our investigation utilizing UB-OC1 cells to elucidate the role of TWIK1 in the cochlear system, we observed TWIK1 expression in the mitochondria of UB-OC1 cells through immunohistochemistry. Moreover, knockdown of TWIK1 expression using shRNA resulted in elevated mitochondrial membrane potential and levels of reactive oxygen species (ROS), along with impaired Nrf2/Keap1/HO-1 pathway in UB-OC1 cells, indicating a potential regulatory role of TWIK1 in oxidative stress. To further substantiate these findings, we utilized cochlear implants from TWIK1-deficient mice and observed an increased susceptibility to oxidative stress compared to the control group. Additionally, we confirmed the expression of TWIK1 in cochlear outer hair cells (OHCs) in TWIK1-BAC-GFP mice. The absence of TWIK1 led to a heightened rate of OHC death and increased vulnerability to noise-induced oxidative stress in a mouse model of NIHL. Consequently, this resulted in accelerated loss of sensory hair cells and severe hearing impairment in TWIK1 KO mice. Overall, our findings provide compelling evidence supporting the crucial role of TWIK1 channels as significant contributors to hearing loss under conditions of oxidative stress.

Keywords: NIHL, hair cell, TWIK1, mitochondria, oxidative stress

P16-9

Identifying mitochondrial matrix protein critical for muscle mitochondrial activity via *In vivo* proximity labeling

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Targeting proximity labeling enzymes to specific cellular locations is a viable strategy for profiling subcellular proteomes. However, these tools have mostly been utilized *in vitro* contexts rather than *in vivo*. Here, we generated transgenic mice expressing a mitochondrial matrix-targeted ascorbate peroxidase (MAX-Tg) to analyze tissue-specific matrix proteomes. Desthiobiotin-phenol labeling of muscle tissues from MAX-Tg mice allowed for efficient profiling of mitochondrial-localized proteins in these tissues. Comparative analysis of matrix proteomes from MAX-Tg muscle tissues revealed differential enrichment of mitochondrial proteins related to energy production in between different muscle groups. Among the proteins enriched in mitochondria, we have identified Reticulon 4 interacting protein 1 (RTN4IP1), also known as Optic Atrophy-10 (OPA10), as a mitochondrial antioxidant NADPH oxidoreductase supporting oxidative phosphorylation activity in the matrix. *Rtn4ip1* KO mice were embryonic lethal, suggesting a critical role for RTN4IP1 in mouse embryonic development. Moreover, muscle specific *Rtn4ip1* deficient mice exhibited smaller body size and decreased lifespan concomitant with aberrant mitochondrial morphology and function. Taken together, our findings reveal RTN4IP1 as a mitochondrial matrix protein

necessary for maintaining mitochondrial oxidative phosphorylation activity in muscle tissue.

Keywords: mitochondria, proximity labeling, mitochondrial matrix-targeted ascorbate peroxidase, RTN4IP1

P16-10

Fatty acid-dependent mitochondrial activity and its regulation by neuronal nitric oxide synthase in hypertensive rat hearts

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Fatty acid (FA)-dependent metabolism is important in maintaining cardiac contractile function. Under disease conditions, FA metabolism shifts from FA to glucose-dependent pathways. Recent research has shown that FA-dependent metabolism is dysregulated in hypertensive (HTN) myocardium. Neuronal nitric oxide synthase (nNOS) regulates cardiac physiology and pathology and is involved in mitochondrial activity through its interactions with mitochondrial complexes. Until recently, the mechanisms of the regulation of mitochondrial complexes by nNOS with FA in HTN remain unclear. Therefore, we aimed to investigate mitochondrial activity with FA supplementation in sham and angiotensin II (Ang II)-induced HTN rat hearts and nNOS regulation of complex-mediated mitochondrial activity under these conditions.
Our results showed that oxygen consumption rate (OCR) and intracellular ATP were increased by palmitoyl-carnitine (PC) or palmitic acid (PA). Furthermore, mitochondrial complex I and complex II (C-I and C-II) activity were increased by PA or PC in sham rat hearts. In HTH, C-I activity was increased but C-II activity was reduced by PC, result in reduced mitochondrial OCR. In the presence of C-II inhibitor (malonate, 30 mM) or C-I inhibitor (rotenone, 5μM), OCR was decreased with PA or PC supplementation both in cardiomyocytes and mitochondrial fraction from sham rat hearts. In HTN, however, malonate did not affect mitochondrial OCR in the presence of PC but OCR was increased with rotenone. Therefore, FA increased mitochondrial activity through enhancing of C-I and C-II activity in sham. By contrast, FA-dependent mitochondrial activity was reduced by C-II downregulation in HTN, despite of the fact that C-I activity was increased by PC. nNOS protein was expressed similarly in sham and HTN LV mitochondrial fraction. Inhibition of nNOS with S-methyl-l-thiocitrulline (SMTC) did not affect OCR or cellular ATP in the presence of PC or PA in sham, but increased OCR in HTN without changing myocardial ATP level. SMTC increased C-I activity only in sham (with C-II activity unaffected), but both C-I and C-II activity were increased by SMTC in HTN. In addition, OCR was increased by SMTC+PC or PA with malonate in sham mitochondrial fraction and cardiomyocyte, but such effects were not observed in the presence of rotenone, indicating that nNOS attenuates C-I-mediated OCR. In contrast, SMTC increased OCR with rotenone pretreatment but not with malonate in HTN, suggesting that nNOS modulates C-II-mediated OCR in HTN. Furthermore, nNOS-derived NO was increased by rotenone in LV myocytes with PA in sham. nNOS-derived NO was partially reduced by malonate with PA in HTN.In parallel, I went on and investigated the effects of nNOS on OCR in atrial myocardium. In atria, OCR was greater in HTN-LA compared to those in sham-LA and PA increased OCR further in sham-LA but reduced it in HTN-LA.Taken together, metabolic dysregulation by nNOS underlie atrial and ventricular remodeling in HTN.

Keywords: hypertension, metabolism, nNOS, mitochondrial complexes, fatty acids

P17-1

Mechanism of arsenic trioxide inhibiting melanoma B16 cells in vitro

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Background: The mechanism of arsenic trioxide (As_2O_3) inhibiting melanoma is not fully understood. In this study, we investigated the effects of As_2O_3 on melanoma B16 cells based on transcriptomic and metabolomic analysis.
Methods: Melanoma B16 cells were co-cultured with As_2O_3 in different concentrations (0μM, 0.002μM, 0.039μM, 0.078μM, 0.156μM, 0.312μM, 0.625μM, 1.25μM, 2.5μM, 5μM, 10μM) for 48h. MTT assay was performed for cell proliferation. Apoptosis, autophagy, and cell cycle were detected by flow cytometry and transmission electron microscopy. Reference transcriptome sequencing and metabolomics analyses were applied on As_2O_3 co-cultured B16 cells. And the signaling pathways involved were verified by Western blot.
Results: We found that As_2O_3 significantly inhibited B16 cell growth in a dose-dependent manner. Both apoptosis and autophagy were induced by As_2O_3 on B16 cell. And cell cycle was stagnated in G1 phase when B16 cells were co-cultured with As_2O_3. Caspase-9, PINK1, Parkin, Beclin-1 and LC3 increased to different extents, but CDK1 decreased in the analysis of reference transcriptome sequencing. Significant increase in glutamic acid, dichlorobenzoic acid, piperidinic acid, lysine and other metabolites were detected by metabolomics and cluster analyses. While the level of thiamine was obvious decrease. Western blot analysis also revealed that As_2O_3 significantly increased the expression levels of Cleaved Caspase-9, PINK1, Parkin, LC3-II/I, and Beclin-1 was increased, and the expression levels of CDK1was dramatically decreased .
Conclusion: As_2O_3 may inhibit the proliferation of B16 cells by synergistic effect of induction of apoptosis, autophagy, cell cycle arrest and as-resulted metabolic changes.

Keywords: arsenic trioxide, melanoma, apoptosis, autophagy, cell cycle

P17-2

Overexpression of NUDT16L1 sustains proper function of mitochondria and leads to ferroptosis insensitivity in colorectal cancer

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Recent studies have shown the potential of ferroptosis inducers in treating various cancer types. However, the lack of a systematic analysis of ferroptosis sensitivity in different cancer types and the unidentified critical regulator determining ferroptosis sensitivity during cancer progression has limited its clinical usage. Here, we identify colorectal cancer as one of the ferroptosis-insensitive cancer types and discover a

novel ferroptosis repressor, NUDT16L1, that contributes to this insensitivity. Notably, NUDT16L1 localizes to the mitochondria to maintain its proper function after ferroptosis inducer treatment in colon cancer cells. Our mouse models of colon cancer demonstrated the critical role of NUDT16L1 in promoting tumor growth. Clinical analyses revealed that NUDT16L1 overexpression specifically in the epithelial cells of colorectal cancer. Furthermore, a specific NUDT16L1 inhibitor demonstrated its therapeutic potential in vitro and in vivo. Our results provide new insights into the crucial role of NUDT16L1 in promoting tumor growth and ferroptosis insensitivity in colorectal cancer and its potential as a therapeutic target. These findings offer a promising avenue for future colorectal cancer treatment research and clinical application.

Keywords: colon cancer, ferroptosis insensitivity, NUDT16L1, mitochondria

P17-3

Novel epigenetic therapeutic target for retinoblastoma, RB1-deficient tumor

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Retinoblastoma is the most common cause of pediatric ocular tumor. It is often accompanied with RB1 gene mutation. This leads to dysregulation of the G_1 checkpoint of the cell cycle causing reduced apoptosis and uncontrolled cell proliferation. Chemotherapies are used for treating retinoblastoma, but many patients undergo enucleation. This emphasizes the need of investigating alternative therapeutic strategies and novel therapeutic markers. This study investigates protein arginine deiminase II (PADI2) as a novel epigenetic target for retinoblastoma, and furthermore, RB1-deficient tumors.

Two retinoblastoma cell lines, Y79 and WERI-Rb1, and orthotopic transplantation mouse model were used. For PADI2 inhibition, a chemical inhibitor, BB-Cl-Amidine, and short hairpin RNA (shRNA) targeting PADI2 were used. The effects of PADI2 inhibition in retinoblastoma cell lines were evaluated with cell cycle assay, cell proliferation assay, and cell apoptosis assay. The upstream and downstream molecular pathways were clarified with western blotting and RT-PCR. Orthotopic transplantation mouse model was prepared by injecting cell lines intravitreally into BALB/c nude mice. The tumor suppression effects were evaluated through visual grading and hematoxylin and eosin (H&E) staining. The expression of PADI2 were evaluated with immunohistochemistry in human and mice tissue and with immunofluorescence assay in cell lines.

The expression of PADI2 in retinoblastoma were enhanced in both human and mouse tissues and retinoblastoma cell lines compared to normal control. Analysis of upstream pathway revealed that the overactivation of E2 promoter factor (E2F) and specificity factor 1 (Sp1) were involved in PADI2 overexpression. The inhibition of PADI2 by both chemical inhibitor and shRNA displayed increased apoptosis, cell cycle arrest, and reduced cell proliferation *in vitro*. Molecular analysis displayed the reduction of phosphorylation of AKT and increased expression of cleaved PARP as the downstream pathway. Results of orthotopic transplantation mouse model showed similar results with significantly suppressed tumor growth under PADI2 inhibition. In addition, BB-Cl-Amidine showed low toxicity *in vivo* promising high feasibility for human translation.

This study investigated the anti-proliferative effects of PADI2 inhibition in RB1-deficient retinoblastoma. The oncogenic function of PADI2 in retinoblastoma and the therapeutic effects of its suppression was both confirmed. Furthermore, this strategy may be expanded to other tumors with RB1 deficiency, one of the major causes of cancer.

Keywords: RB1-deficient tumor, epigenetics, retinoblastoma, AKT phosphorylation, orthotopic transplantation mouse model

P17-4

Insulin resistance-associated neddylation impairment promotes accelerated tumor migration

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Background: Malfunction in insulin, characterized by hyperinsulinemia and insulin resistance, extends beyond its association with obesity and diabetes, encompassing an elevated risk for various cancer types. While the correlation between hyperinsulinemia and tumor aggressiveness has been proposed, the underlying mechanisms driving this remain largely unexplored. Further investigation is needed to understand the intricate relationship between insulin dysfunction and cancer progression, offering potential insights for personalized treatment approaches.

Methods: The involvement of neddylation in insulin signaling among cancer patients was explored using proteomics and informatics. To assess cell migration, insulin resistance under the condition of neddylation blockade was induced using various migration assays, si-RNA transfection system, and the 3D cell-culture system. Molecular events driving cell migration were investigated using quantitative real time-PCR, immunoprecipitation and immunoblotting. Clinical data was obtained by analyzing ovarian cancer tissues from patients with and without type 2 diabetes mellitus using immunohistochemistry.

Results: Our findings revealed that prolonged insulin exposure, coupled with neddylation inhibition, facilitated aggressive cancer cell migration. This effect was mediated by the upregulation of IRS1 and IRS2, leading to the activation of the PI3K/AKT pathway. Moreover, we discovered a direct interaction between NEDD8 and both IRS1 and IRS2, implicating the role of neddylation in the regulation of their protein stability. We also demonstrated that neddylation precedes the process of ubiquitination for IRS1 and IRS2, shedding light on the mechanisms governing their degradation. Significantly, we identified C-CBL as the neddylation E3 ligase responsible for the neddylation of both IRS1 and IRS2. Supporting our experimental findings, clinical data analysis demonstrated increased expression levels of IRS1 and IRS2, along with decreased expression of NEDD8, in ovarian cancer tissues from patients with type 2 diabetes mellitus.

Conclusion: Altogether, our data provides compelling evidence for the involvement of neddylation in cancer cell migration under conditions

of insulin resistance. This highlights the potential significance of neddylation as a therapeutic target for cancer treatment. However, careful consideration is required regarding the use of neddylation inhibitors as anticancer agents in patients with obesity or diabetes. The interplay between neddylation, insulin signaling, and cancer progression necessitates further investigation to fully understand the underlying molecular mechanisms, which holds promising prospects for the development of targeted therapies in insulin resistance-associated cancers.

Keywords: insulin resistance, insulin receptor substrates, neddylation, cancer cell migration, posttranslational modification

P17-5

Depletion of UBA6 affects vesicle trafficking by increase of endosomes and exosomes

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Background: UBA6 is a member of E1 ubiquitin ligase to initiate ubiquitination. The ubiquitination has various cellular functions including apoptosis, cell cycle, protein degradation, and biogenesis of organelles. In addition, ubiquitination activity induces endosome formation and lysosome sorting. However, the study of endosome and exosome trafficking with UBA6 has not been fully demonstrated. Thus, we evaluated the relationship between UBA6 and endosome/exosome trafficking.
Methods: To demonstrate the effect of UBA6, endosomal/lysosomal and autophagic proteins was measured through western blotting. To detect the location of intracellular vesicles, immunofluorescence was measured with vesicular proteins such as Ra7 and CD63. Intracellular and exosomal vesicles was measured by transmission electron miscopy to confirm the changes of vesicular density.
Results: The siRNA of UBA6 decreased lysosomal proteins including TPRML1 and TPC2, however, the proteins of autophagosomes and even intracellular protein level have no changes. The intracellular endosomal and exosomal vesicles contents and the secretion of exosomes were increased by the knockdown of UBA6.
Conclusion: The depletion of UBA6 increases exosome exocytosis, suggesting that the inhibition of UBA6 may involve the modulation of intracellular vesicle trafficking.

Keywords: UBA6, endosome, exosome, lysosome, TRPML1

P17-6

Propyl gallate and N-acetyl cysteine effectively protect Calu-6 and A549 lung cancer cells from oxidative stress caused by exogenous H_2O_2

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Exogenous H_2O_2 is often utilized to simulate oxidative stress in cells. The present study investigated effects of propyl gallate (PG) and N-acetyl cysteine (NAC) as well-known antioxidants on cell growth and cell death as well as reactive oxygen species (ROS) and glutathione (GSH) levels in H_2O_2-treated Calu-6 and A549 lung cancer cells. Treatment with 100 mM H_2O_2 efficiently inhibited growth of both lung cancer cells at 24 h and induced their cell death via apoptosis and/or necrosis. H_2O_2 also induced G1 arrest of the cell cycle and significantly triggered a loss of mitochondrial membrane potential (MMP; DYm). Treatment with PG (1 mM ~ 50 mM) and NAC (2 mM) generally decreased the growth inhibition and G1 phase arrest of H_2O_2-treated Calu-6 and A549 cells. Both agents also significantly reduced the number of annexin V-fluorescein isothiocyanate (FITC)-positive cells and MMP (DYm) loss of H_2O_2-treated cells. Exogenous H_2O_2 increased ROS levels including $O_2^{\cdot-}$ in both lung cancer cells at 24 h. Some doses of PG and NAC effectively decreased ROS levels including $O_2^{\cdot-}$ in H_2O_2-treated Calu-6 and A549 cells. Moreover, H_2O_2 induced GSH depletion in both Calu-6 and A549 cells at 24 h. Some doses of PG and NAC significantly suppressed GSH depletion in H_2O_2-treated Calu-6 and A549 cells. PG appeared to increase GSH levels in these cells. In conclusion, PG and NAC can efficiently protect Calu-6 and A549 lung cancer cells from oxidative stress caused by exogenous H_2O_2.

Keywords: lung cancer cells, N-acetyl cysteine, propyl gallate, reactive oxygen species, glutathione

P17-7

The effects of diethyldithiocarbamate, 3-amino-1,2,4-triazole, or buthionine sulfoximine on cell growth and death as well as redox status in H_2O_2-treated lung cancer cells

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Exogenous H_2O_2 is often used to induce oxidative stress in cells. The present study investigated the effects of diethyldithiocarbamate (DDC; a superoxide dismutase inhibitor), 3-amino-1,2,4-triazole (AT; a catalase inhibitor), and buthionine sulfoximine [BSO; a glutathione (GSH) synthesis inhibitor] on cell growth and death, as well as reactive oxygen species (ROS) and GSH levels in H_2O_2-treated Calu-6 and A549 lung cancer cells. Treatment with 75 mM or 100 mM H_2O_2 inhibited the growth and promoted the death of the lung cancer cells at 24 h. H_2O_2 also induced G1 arrest of the cell cycle and the loss of mitochondrial membrane potential (MMP; DYm). BSO increased growth inhibition and G1 phase arrest in H_2O_2-treated Calu-6 cells. It also increased the number of annexin V-fluorescein isothiocyanate (FITC)-positive cells and MMP (DYm) loss in the cells. In addition, BSO slightly increased cell death in H_2O_2-treated A549 cells. Neither DDC nor AT significantly affected cell growth or death in H_2O_2-treated lung cancer cells and further increased ROS levels including $O_2^{\cdot-}$ in the cells at 1 h and 24 h. BSO strongly enhanced $O_2^{\cdot-}$ levels in H_2O_2-treated Calu-6 cells at 24 h. Moreover, H_2O_2 induced GSH depletion in Calu-6 and A549 cells at 24 h. BSO significantly increased GSH depletion in H_2O_2-treated Calu-6 cells. In conclusion, among DDC, AT, and BSO, BSO enhanced cell death by increasing $O_2^{\cdot-}$ levels and GSH depletion in H_2O_2-treated lung cancer cells, especially Calu-6 cells.

Keywords: lung cancer cells, BSO, Cell death, reactive oxygen species, glutathione

P17-8

Humanin promotes glioblastoma progression through integrin αV-TGFβ axis

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Background: Glioblastoma multiforme (GBM) is a highly challenging cancer to treat due to intratumoral heterogeneity, particularly driven by a subpopulation of stem cells known as glioblastoma stem cells (GSCs), which are responsible for tumor recurrence and therapy resistance. Therefore, understanding the role of GSCs is crucial for developing more effective treatment strategies. However, the molecular mechanism underlying the spread of GSCs remains largely unknown. This study aims to investigate the impact of Humanin, a peptide derived from mitochondria, on GBM progression and its correlation with patient outcomes.

Methods: A panel of seven GSCs (448T, X01, X02, 528, 0502, 83, and 1123) and three small cell lung cancer cell lines (H69, H82, and H889) were utilized in this project. Humanin, scrambled Humanin, and other humanin-like peptides were administered to the cells for 24 hours before conducting various techniques such as western blotting, qPCR, co-immunoprecipitation, and immunofluorescence. Cell attachment was quantified using a crystal violet assay. For the in vivo experiment, 83 cells mixed with either Humanin or scrambled Humanin were injected intracranially into the skulls of five-week-old nude mice. The mice's condition was monitored regularly, and any abnormal symptoms were recorded before sacrificing them. The mice's brains were then collected for further analysis, including H&E staining and immunohistochemistry (IHC). The survival of the mice was recorded and analyzed using Kaplan-Meier plots.

Results and Discussion: Treatment with exogenous Humanin was found to enhance the attachment of GSCs by activating the integrin αV-TGFβ axis. Specifically, Humanin activated the signaling of integrin αV and its downstream targets, resulting in the strengthening of the focal adhesion complex, increased filopodia formation, and changes in cell shape. Furthermore, Humanin-induced attachment led to crosstalk between integrin and TGFβ, activating the canonical TGFβ signaling pathway and promoting cell migration and invasion. In line with these findings, in vivo data demonstrated the pro-tumoral effect of Humanin in GBM, characterized by poor survival, increased invasiveness, and enhanced angiogenesis in the Humanin-treated group. These results suggest the potential for developing therapeutic approaches targeting Humanin's functions for the treatment of GBM.

Conclusion: The role of the mitochondrial peptide Humanin in cancer remains largely unknown. However, this study proposes that Humanin treatment enhances tumor progression through the activation of integrin αV and TGFβ signaling, resulting in increased cell migration and reduced survival in tumor-bearing mice. By gaining a better understanding of the mechanisms underlying Humanin's effects on GSCs, further research is needed to fully comprehend its biological significance and assess its potential as a targeted therapeutic approach for GBM.

Keywords: Humanin, GBM, TGF, integrin

P17-9

Development of a high-throughput system for advanced functional cell analysis within immuno-oncology

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Chemo- and radio-therapy are still the dominant treatment types for cancer, but advancing therapies such as immuno-therapy have emerged as promising tools. In general, identifying T cells that kill cancer cells in vivo or monitoring CAR-T cell activity, is critical to the development of successful cell therapies.

The here developed label-free AtlaZ immune cell killing assay allows to investigate cancer cells in a 6 x 96-well throughput format. The platform can be e.g. applied to measure rate of killing to predict in vivo activity. Besides that, the technological methodology used here, electrical impedance spectroscopy (EIS), allows to gain a deeper understanding of cells to access kinetic and phenotypic information.

The impedance of planar gold-film electrodes that are used as growth substrate for cells reveals changes in electrode coverage or cell behavior. Real-time impedance data provide insights in various cell phenotypes, such as cell morphology, proliferation, lateral migration or cytotoxicity even over prolonged periods of time. Advanced information content is obtained by using multi- frequency impedance readouts (0.1 kHz – 100 kHz). For example, high frequency impedance is highly sensitive to differences in cell-confluency, making it useful for measuring cell growth or proliferation rates and cytotoxicity, whereas low frequency impedance detects changes in the space under or between cells and therefore enables barrier function and cell adhesion quantification.

We used A549 cell line which is a widely utilized epithelial lung adenocarcinoma cell line that was derived from a primary lung tumor. Effector cells co-cultured in our here executed killing assay were purified human cytotoxic T- lymphocytes expressing CD8 (cluster of differentiation 8), a co-receptor for the T-cell receptor (TCR).

Investigating A549 cells, we found that after approx. t = 27 h the cytolysis of A549 cells gradually increases and reaches a maximum of 37%, 48%, 59% and 57% in the presence of the target to effector cell ratio 1:2, 1:1, 2:1 and 3:1, respectively. Uninterrupted attachment and growth of A549 cells was observed in wells with only A549 cells. The Kill Time 50 values were calculated to investigate at what timepoint and which ratios of effector cells killed the cancer cells to 50%. We found that 50% of A549 cells were killed after approx. 13 h (E:T = 1:1) or 6 h (E:T = 2:1 and 3:1).

The platform developed here, AtlaZ, is a quantitative live-cell analysis system and allows for cellular research on cell adhesion and proliferation, cytotoxicity and cell viability, label-free and in real-time. Recordings can be performed in up to six 96-well plates simultaneously or independently. Electrical impedance spectroscopy in combination with the throughput allows for a so far unmet quantity and richness of information which can be gained from cells, through the potential to access multiple kinetic and phenotypic information from *in vitro* 2D or 3D cell cultures.

Keywords: innovation, cancer research, CAR T, immuno-oncology, GPCR

Poster

P17-10

Loss of ATM activity in macrophages impairs dacarbazine-induced phagocytosis of melanoma cells

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Chemotherapeutic agent-based therapy is a common strategy for treating metastatic melanoma. The DNA damage response elicited by chemotherapy regulates the interplay between tumor cells and the immune microenvironment, which can affect the response to chemotherapy. However, the basic mechanisms of chemotherapy-induced responses, particularly in tumor-associated macrophages, remain poorly defined. In this study, we showed that dacarbazine (DTIC), a chemotherapeutic drug approved as a treatment for melanoma, increased reactive oxygen species (ROS) production in RAW264.7 and phorbol 12-myristate 13-acetate (PMA)-differentiated THP-1 cells, and enhanced the macrophage phagocytosis of melanoma cells, while the inhibition of ROS production impaired DTIC-induced macrophage phagocytosis. We also found that the inhibition of ataxia telangiectasia mutated (ATM), the key kinase involved in the initiation of DNA damage response, reduced the DTIC-treated macrophage phagocytosis of melanoma cells. Furthermore, we observed that in the murine model of melanoma, the phagocytosis of tumor cells was increased in macrophages with activated ATM. Our results demonstrated that elevated ROS production and intact ATM activity in macrophages contributed to the phagocytic clearance of melanoma cells.

Keywords: ataxia telangiectasia mutated (ATM), DNA damage, phagocytosis, reactive oxygen species (ROS), chemotherapy

P17-11

The role of PAK4 in the progression of hepatocellular carcinoma and its implications for immunotherapy

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P21 activated kinase (PAKs) are known to contribute to changes in cell morphology and function. Among the various members of the PAK family, only PAK4 protein has been shown to be overexpressed in cancer cells, and upregulation of PAK4 is associated with tumor development. Although PAK4 is highly expressed in liver cancer, the role of PAK4 in the immune response in liver cancer has not yet been clearly understood. Our study found that PAK4 knockdown inhibited carcinogenesis, including a reduction in cell proliferation, invasion, migration, and the induction of apoptosis in liver cancer cells. In addition, we confirmed that the expression of PD-L1 was significantly reduced when PAK4 was suppressed. These findings suggest that PAK4 is involved in the progression of liver cancer cells by regulating the immune checkpoint. Therefore, it is necessary to determine how PAK4 affects the PD-1/PD-L1 pathway and modulates immune cells in response to immune checkpoint blockade therapy.

Keywords: PAK4, PD-L1, liver cancer

P17-12

Innovative therapeutic approaches that target HN1 and combined it with PD-1 immune checkpoint blockade in hepatocellular carcinoma

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There has been no research conducted on the interaction between PD-L1 and hematological and neurological expressed 1 (HN1) and their effects on T cell activation in hepatocellular carcinoma (HCC). This study, we investigated the interaction between PD-L1 and HN1 in HCC and their impact on the progression of HCC. Our findings revealed that the genetic deletion of HN1 in tumor cells lead to a suppressed tumor progression in immune-deficient xenografts. In addition, the genetic deletion of HN1 in hepa1-6 cells resulted in the upregulation of PD-L1, leading to G1 phase arrest of T cells and decreased activity of co-cultured T lymphocytes. Furthermore, we made the discovery that HN interacts with GSK3β, promoting its activation through the dephosphorylation of serine 9 (S9), inducing polyubiquitination of PD-L1. Moreover, our study demonstrated that targeted therapy against HN1 in an immunocompetent (C57BL/6) mice model resulted in the attenuation of anti-tumor CD8+T-cell immunity. Overall, this study shed light on the relationship between HN1 and immune surveillance in HCC and propose novel therapeutic strategies targeting HN1 and PD-1 immune checkpoint blockade to enhance the efficacy of immune-based therapies in HCC.

Keywords: hematologic and neurological expression 1(HN1), programmed death ligand 1(PD-L1), hepatocellular carcinoma (HCC)

P17-13

Melanophilin-induced primary cilia drive pancreatic cancer metastasis under glutamine deficiency

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Pancreatic ductal adenocarcinoma (PDAC) cells rely on glutamine (Gln) to sustain their metabolic homeostasis and proliferation. However, high consumption of Gln leads to metabolic stress that causes poor prognosis including metastasis in PDAC. Primary cilium acts as a cellular antenna that senses and transmits environmental signals into the cell to initiate proper responses. PDAC cells are devoid of primary cilia; however, ciliated PDAC cells link to chemoresistance in PDAC, implying the pathological roles of primary cilia in tumorigenesis. In this study, we demonstrated that primary cilia contributed to PDAC metastasis when cells adapted to Gln deprivation (in short as -QQ cells). Mechanistically, melanophilin (MLPH) was upregulated and localized to the base of primary cilia in -QQ cells. In addition, high MLPH levels were observed in human PDAC tumors and associated with poor survival rates. Importantly, MLPH-mediated primary ciliogenesis is crucial for PDAC cell invasion 2-D cell culture, in PDAC organoids, and in a mouse model of metastatic pancreatic cancer. By using kinase array screening, we found that phospholipase C gamma 1 (PLCγ-1) was upregulated and activated to promote epithelial-mesenchymal transition (EMT) and metastasis of -QQ PDAC cells. Disruption of primary cilia alleviated PLCγ-1 upregulation, EMT, and metastasis. These finding not only decipher the link between Gln deficiency and PDAC metastasis but uncover a novel pathophysiological function of primary cilia in PDAC metastasis.

Keywords: primary cilia, metastasis, melanophilin, phospholipase C gamma 1, nutrient deprivation

P17-14

WISP-1 inhibits the migration and invasion of lung cancer cells and cancer-associated fibroblasts preventing lung cancer metastasis

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Wnt-induced signaling protein 1 (WISP-1) exerts oncogenic or tumor-suppressive effects in different tumor types. In the present study, we investigated whether and how WISP-1 modulates migration and invasion of cancer cells and CAFs. 344SQ cells and CAFs isolated from the lung tumors of Kras-mutant (KrasLA1) mice were used for Transwell migration and invasion assays. Western blotting and real-time quantitative PCR were used to assess TGFβ1 signaling pathways. For in vivo study, WISP-1 was administered via intratumoral injection following subcutaneously injection of 344SQ cells into syngeneic (129/Sv) mice. Treatment with rWISP-1 at the concentrations at 5 and 10 ng/ml or 0.75-3 ng/ml, respectively, inhibited the migration and invasion of 344SQ cells and CAFs in a dose-dependent manner. rWSIP-1 also blocked TGF-b1 signaling pathways, including Smad2/3, ERK, Src, p38 MAP kinase, FAK, and Akt as well as matrix metalloproteinases (MMP2)/MMP12 mRNA and protein expression in 344SQ cells and CAFs. Utilizing blocking antibodies against integrin α5, αv, β1, β3, or β5, our study indicates that integrin αvβ3 in 344SQ cells and αvβ5 in CAFs act as functional receptors for WIPS-1. In addition, administration of rWISP-1(12.5 or 25 μg/kg) suppressed tumor growth, migration and invasion of tumor cells and CAFs, and development of the metastasis. Collectively, these data demonstrate that WISP-1 plays critical roles for preventing lung cancer progression and metastasis.

Keywords: WISP-1, lung cancer cells, CAFs, metastasis

P17-15

Notch1-WISP-1 signaling in cancer-associated fibroblast stimulated with apoptotic cancer cells inhibits cancer cell growth

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Cancer-associated fibroblasts (CAFs) and apoptotic cancer cells modulate cancer progression and metastasis in the tumor microenvironment (TME). Here, we demonstrate that treatment with conditioned medium (CM) from CAFs exposed to apoptotic cancer cells suppresses cell proliferation and enhances apoptosis of lung cancer cells. Pharmacologic inhibition of Notch1 activation by LY3039478 or siRNA-mediated Notch1 silencing in CAFs reversed the antiproliferative and proapoptotic effects of CM from CAFs exposed to apoptotic 344SQ or A549 cells. Similarly, knockdown of Wnt-induced signaling protein 1 (WISP-1) in CAFs by its specific siRNA or neutralizing the CM with anti-WISP-1 antibodies also reversed the antiproliferative and proapoptotic effects. Importantly, administration of CM from CAFs exposed to apoptotic 344SQ cells (ApoSQ-CAF CM) suppressed tumor weight and volume, whereas WISP-1-immunodepleted ApoSQ-CAF CM reversed anti-tumor growth effects. Thus, treatment with CM from CAFs exposed apoptotic lung cancer cells could be an effective therapeutic approach against tumor growth.

Keywords: CAFs, apoptotic lung cancer cells, tumor growth, WISP-1, Notch1

P17-16

Cancer cells promote lipolysis of adipocyte derived stem cells by using a cytokine to obtain free fatty acids for cancer proliferation and migration

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In our previous research, it has shown that coculture of cancer cells with human adipocyte derived stem cells (hADSCs) has resulted in enhanced proliferation and metastasis when cancer cells consume free fatty acids (FFAs) derived from hADSCs. Because higher concentration of FFAs has been found from the conditioned media (CM) when hADSCs are cocultured with cancer cells then when it cultured alone, our study has focused on the effect of cancer as further study. As a result, we found cancer cells secrete a specific cytokine to enhance lipolysis of hADSCs. The cytokine upregulates genes which are related to lipolysis by increasing the expression of a specific protein. On the other hand, it is shown that lipolysis and metastasis of cancer cells on a 3D organoid chip are suppressed when the cytokine from cancer cells and the protein of hADSCs are inhibited. Taken together, it seems like cancer cells control hADSCs via the cytokine to derive FFAs from hADSCs and consume the FFAs for migration. Therefore, this finding supports poor prognosis of cancer patients who have obesity.

Keywords: adipocyte derived stem cells, cancer proliferation, cancer metastasis, chemokine, lipolysis

P17-17

The effect of necrosis on endoplasmic reticulum stress and unfolded protein response in glioblastoma

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Glioblastoma multiforme (GBM) is the most aggressive and malignant form of glioma, in addition to being the most common type of primary brain tumor in adults. One of the remarkable characteristics that differentiate GBM from other types of glioma is the presence of necrosis. Previous studies have focused on the molecular mechanisms involved in necrosis formation in GBM; however, the effect of necrosis on cancer cells after necrosis formation remains unclear. Recent studies conducted in our lab have revealed that exposure to necrosis induces the secretion of several chemokines, such as interleukin (IL)-8, MCP1, and MIP-3α, which promote GBM migration, invasion, and microglial infiltration. Under stressful conditions, such as the accumulation of misfolded

proteins, glucose deprivation, or hypoxia, cancer cells evoke the unfolded protein response (UPR) to adapt to these situations. In this study, we tested whether necrosis is associated with endoplasmic reticulum (ER) stress and the UPR in GBM. Furthermore, we examined the effect of necrosis on GBM progression by analyzing the signaling pathways and molecules involved in ER stress/UPR.

Keywords: glioblastoma multiforme, necrosis, unfolded protein response, endoplasmic reticulum stress

P17-18

Triple-negative breast cancer tumor-targeted treatment using RNA nanoparticles containing anti-miR-21 and the α9-nAchR aptamer

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About 10–20% of breast cancer patients have triple-negative breast cancer (TNBC), characterized by tumors that test negative for increased HER2 protein, progesterone receptors, and estrogen receptors. Because TNBC is more aggressive with a poorer prognosis and lacks cell markers as a treatment target, getting the diagnosis is terrifying. The α9-nicotinic acetylcholine receptor (nAchR), abundantly expressed in TNBC, can be used as a biomarker to identify the disease and guide treatment. In this study, we built RNA nanoparticles with an anti-α9-nAChR RNA aptamer as a targeting ligand and an anti-miR21 as a therapeutic module using thermodynamically stable three-way junction (3WJ) pRNA as the core. With the aptamer and miRNA attached to two separate strands of the 3WJ, we looked into and compared the configurations of the two RNA nanoparticles. Compared to attaching to the 5' end of the C-strand, it was discovered that connecting to the 3' end of the B-strand (3WJ-B-α9-apt-Alexa) produced a greater binding affinity. Additionally, after systemic injection in mice, 3WJ-B-α9-apt-Alexa bound to TNBC patient-derived xenograft more effectively than 3WJ fluorescent RNA nanoparticles (3WJ-Alex), displaying remarkable selectivity with minimal to no accumulations in healthy organs. Moreover, the 3WJ-B-a9-nAChR-aptamer RNA nanoparticles containing anti-miR21 locked nucleic acid (3WJ-B-α9-apt-anti-miR21) dramatically reduced PDX-TNBC tumor development and promoted cell death. When the 3WJ-B-a9-nAChR-aptamer was employed, there was a decrease in the expression of miR21 and an increase in the expression of PTEN and PDCD4. Additionally, neither biochemical blood tests nor a histological examination of the treated mice's main organs revealed any abnormal alterations. In conclusion, the preclinical testing showed that the 3WJ-B-α9-nAChR-aptamer RNA nanoparticles developed in this work could effectively deliver therapeutic anti-miRNA21 for treating TNBC.

Keywords: α9-nicotinic acetylcholine receptor, RNA aptamer, patient derived xenograft, microRNA 21

P17-19

The inhibitory effects of resveratrol on human tongue carcinoma Tca8113 cells and its mechanism

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Background: Resveratrol, a type of polyphenol compound derived from plant extracts, has shown antitumor activity in different types of cancer cells. However, its antitumor effect on human tongue squamous carcinoma cells (TSCC) is not clear. We herein investigated the antitumor effects of resveratrol on TSCC and elucidated the underlying mechanisms.
Methods: Cell proliferation, apoptosis and adhesion, migration, and invasion of TSCC cells (Tca8113) were evaluated after resveratrol treatment using Sulforhodamine B assay and Boyden chamber assay. The associated regulatory mechanisms were examined. Gelatin zymography assay was performed for the evaluation of matrix metalloproteinase (MMP) activity, along with immunohistochemistry assay and western blotting analysis.
Results: Our data indicated that resveratrol inhibited Tca8113 cell proliferation, adhesion, migration, and invasion and promoted cell apoptosis. They were accompanied by down-regulation of Akt and IKK signaling pathways, reduction of MMP-2 and MMP-9 activities, and NF-κB p65 nuclear translocation.
Conclusion: Resveratrol has antiproliferative, proapoptotic, and antimetastatic effects on TSCC *via* Akt/IKK mediated NF-κB mechanism, and potentially could be a therapeutic agent against tongue cancer.

Keywords: resveratrol, tongue cancer, human tongue squamous carcinoma cells, metastasis, molecular mechanisms

P17-20

HDAC inhibitor and 5-fluorouracil combination therapy: A novel approach to gastric cancer treatment

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Histone deacetylase (HDAC) inhibitors cause apoptosis and suppress cancer cell proliferation, metastasis, and tumorigenesis in various types of cancers. The Hippo signaling pathway plays an important role in organ development and tissue regeneration. Dysregulated Hippo signaling is linked to cancer, making this pathway a promising target for treatment. In this study, we report that combining the HDAC inhibitor poanobinostat and 5-fluorouracil (5-FU) synergistically induces apoptosis in gastric cancer. Panobinostat and 5-FU suppressed cell viability more efficiently than either treatment alone, as evidenced by increased protein levels of cleaved-PARP and cleaved caspase-9. Panobinostat and 5-FU also significantly repressed cell migration by targeting E-cadherin, MMP-9, and vimentin. In addition, panobinostat potently enhanced the antitumor action of 5-FU by inactivating the Akt signaling pathway and activating the Hippo signaling pathway (MST1 and 2, sav1 and MOB1), thereby decreasing Yap protein. In a xenograft animal model, combining panobinostat and 5-FU treatment significantly inhibited the

progression of gastric cancer by inactivating Akt pathway and increasing the Hippo pathway. Therefore, panobinostat effectively potentiates the anti-tumor efficacy of 5-FU, as combination treatment showed significantly more anti-tumor potential than 5-FU alone. The combination treatment with panobinostat and 5-FU is therefore considered to be a valuable complementary chemotherapy agent for the future.

Keywords: 5-FU, HDAC inhibitor, apoptosis, gastric cancer

P17-21

Treatment of EGFR-mediated tumors via lysosome activity regulation

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The lysosome is a membrane-enclosed organelle that functions as an essential part of the cell's digestive system. This organelle contains over 60 types of hydrolases that can break down biological polymers such as proteins, carbohydrates, lipids, and nucleic acids. These enzymes require an acidic pH for optimal function, achieved by using ATP hydrolysis to pump protons against their electrochemical gradient into the lysosome by the vacuolar H^+ ATPase (V-ATPase).
The biogenesis of lysosomes is known to be controlled by the coordination of the regulatory gene network and the lysosomal expression, which is governed by the nuclear translocation of transcription factor EB (TFEB). Lysosomes are an essential component of the inner membrane system and participate in numerous cell biological processes, such as macromolecular degradation, antigen presentation, intracellular pathogen destruction, plasma membrane repair, exosome release, cell adhesion/migration, and apoptosis.
The primary function of lysosomes is to digest extracellular material that has been internalized by endocytosis and intracellular components that have been sequestered by autophagy. They recycle the unwanted cellular material as energy, providing a nutrient source for maintaining cellular homeostasis.
Lysosomal activity affects the tumor microenvironment, making the tumor cells use energy more efficiently, and it is known to be increased compared to neighboring normal tissues. However, recent studies have shown that increased lysosomal activity can lead to downregulation of Receptor tyrosine kinase (RTK) activity in cancer cells[3]. RTK activity frequently occurs with mutations in EGFR in various human cancers. Thus, EGFR is currently a target for several cancer therapies. Recently, studies on EGFR-mediated tumors through lysosome regulation have been continuously conducted. According to our previous study, it has been found that lysosome inactivation suppresses the degradation of RKT, such as EGFR, and increases the expression of EGFR.
EGFR is expressed in various human tumors, including gliomas and carcinomas of the lung, colon, head and neck, pancreas, breast, ovary, and kidney. Mutations, gene amplification, and protein overexpression of various elements of this pathway contribute to carcinogenesis and impact prognosis. Alterations within the EGFR signaling cascade, such as gene mutations, gene amplification, and protein overexpression, have been shown to contribute to colorectal carcinogenesis.
In this study, we will down-regulate the expression of EGFR by activating the lysosome in DLD-1, a colorectal cancer cell line. Lysosome was activated by overexpression of V-ATPase using the lentiviral system. In vitro experiments confirmed that the degradation of EGFR was actively performed in the lysosome-activated experimental group. It was established to inhibit cancer cells' proliferation, survival, and differentiation.
Targeting lysosomes may be a new approach to overcoming EGFR-mediated cancers.

Keywords: lysosome function, lysosome activation, RTK, EGFR, colon Cancer

P17-22

Paeonia japonica inhibits tumor growth in the mouse CT-26 colon tumor model

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Paeonia japonica has been used as a traditional medicine to treat a variety of ailments, including liver disease. However, the inhibitory effect of *Paeonia japonica* on colon cancer has not been fully understood. The purpose of this study was to evaluate the effect of *Paeonia japonica* on colon cancer. The CT-26 cell line was used in vitro study whether *Paeonia japonica* has the effect of treating cancer. After that $2*10^6$ CT-26 cells were injected into the flank of mice for in vivo experiments. Mice were randomly divided into groups receiving *Paeonia japonica* (100 mg/kg) as a positive control or PBS as a negative control. We evaluated the effect of *Paeonia japonica* on colon cancer, measuring tumor reduction in mice. Furthermore, to find a possible explanation for the anticancer effect of *Paeonia japonica*, we evaluated the effect of *Paeonia japonica* on the levels of ERK and AKT in tumors. The results showed that mice treated with *Paeonia japonica* exhibited measurable clinical signs, including a reduction in tumor size. In addition, *Paeonia japonica* suppressed the expression levels of ERK and AKT in tumors. Taken together, the results of this study suggest that *Paeonia japonica* may help treat colon cancer.

Keywords: Paeonia japonica, CT-26 cell, colon cancer, mouse model

P17-23

A novel design of tri-layer coating membrane for biliary stent applications: a promising approach for controlled delivery of paclitaxel with anti-sludge advantage

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Here we developed a novel biliary stent coating material that composed of tri-layer membrane with dual function of sustained release of PTX anticancer drug and antibacterial effect. The advantages of the electrospinning technique have to be considered for the uniform distribution of PTX drug and controlling the release profile. Furthermore, film cast method to make AgNPs-immobilized PU film to direct the release toward tumor side and suppress the biofilm formation. The in vitro antibacterial of our samples was conducted against Gram-positive and Gram-negative bacteria species showing excellent antibacterial effect compared to control sample. The in vitro drug release study confirms the sustained release of PTX from the tri-layer membrane and the release profile fitted first order with correlation coefficient (R2 = 0.98) furthermore, the release mechanism was studied using Korsmeyer–Peppas model revealing the release mechanism follows Fickian diffusion. This novel tri-layer membrane may prolong the stent patency in clinical application.

Keywords: paclitaxel, drug delivery, electrospinning

Poster

P17-24

NIR-responsive composite nanofibers for chemo-phototherapy from controlled drug release to *in vitro* anticancer synergism

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The combination of chemotherapy and hyperthermia has gained significant attention in the local treatment of cancer after surgical resection. Pyrrole is an effective photothermal agent that can induce a temperature increase in the surrounding medium by absorbing near-infrared radiation (NIR). In this study, we used electrospinning technique to prepare nanofibers with poly(ε-caprolactone) (PCL) and poly (d,l-lactic-co-glycolic acid) (PLGA). Pyrrole was then attached to the surface of the PCL-PLGA fiber mats through in situ polymerization at different concentrations of 0.2, 0.4, and 0.6 M. The morphology and physiochemical properties were confirmed through various analyses such as SEM, EDX, FT-IR, and XRD. The NIR laser irradiation resulted in a local temperature rise that depended on the pyrrole concentration, which was observed using a FLIR camera. For hyperthermia effects, a pyrrole concentration (0.06 M) was used for *in vitro* drug release and cell viability assays because it raised the local temperature to about 45°C under NIR irradiation (2 W/cm2, 3 min). *In vitro* drug release studies revealed that NIR irradiation increased the diffusion rate of doxorubicin (DOX) by raising the environmental temperature above the glass transition temperature of PLGA. Furthermore, *in vitro* cytotoxicity experiments confirmed that the PCL-PLGA-DOX/PPy fiber mats demonstrated an enhanced inhibitory effect against CT26 and MCF7 cells by combining hyperthermia and chemotherapy.

Keywords: chemotherapy, hyperthermia, PLGA, drug release, cytotoxicity

P17-25

Doxorubicin and panobinostat: A synergistic combination for apoptosis in colorectal cancer

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Colorectal cancer is the third most common cancer worldwide and the second leading cause of cancer death. Doxorubicin is an effective antineoplastic agent used to treat colorectal cancer, but its resistance is a major obstacle to treatment. This study investigated the efficacy of panobinostat, a histone deacetylase (HDAC) inhibitor, in combination with doxorubicin in colorectal cancer. Panobinostate and doxorubicin combination treatment more effectively inhibited the cell viability and colony formation ability of HCT116 and SW480 cells than either treatment alone. The combination treatment also induced significant apoptosis, as evidenced by increased levels of cleaved caspase-9 and poly ADP-ribose polymerase (PARP). These findings suggest that panobinostat can sensitize doxorubicin-resistant colorectal cancer cells to apoptosis.

Keywords: doxorubicin, panobinostat, cell death, colorectal cancer cells

P17-26

Cyclosporin A inhibits prostate cancer growth through suppression of E2F8

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The treatment of advanced prostate cancer remains a formidable challenge due to the limited availability of effective treatment options. Therefore, it is imperative to identify promising druggable targets that provide substantial clinical benefits and to develop effective treatment strategies to overcome therapeutic resistance. CsA showed an anticancer effect on prostate cancer in cultured cell and xenograft models. E2F8 was identified as a master transcription factor that regulates a clinically significant CsA-specific gene signature. The expression of E2F8 increases during prostate cancer progression, and high levels of E2F8 expression are associated with a poor prognosis in patients with prostate cancer. Additionally, MELK was identified as a crucial upstream regulator of E2F8 expression through the transcriptional regulatory network and Bayesian network analyses. Knockdown of E2F8 or MELK inhibited cell growth and colony formation in prostate cancer cells. High expression levels of E2F8 and androgen receptor (AR) are associated with a worse prognosis in patients with prostate cancer compared to low levels of both genes. The inhibition of E2F8 improved the response to AR blockade therapy. These results suggest that CsA has potential as an effective anticancer treatment for prostate cancer, while also revealing the oncogenic role of E2F8 and its association with clinical outcomes in prostate cancer. These results provide valuable insight into the development of therapeutic and diagnostic approaches for prostate cancer.

Keywords: prostate cancer, oncogene, E2F8, cyclosporin A, prognosis

P17-27

Development and evaluation of nano-emulsion containing curcumin for transdermal delivery to improve therapeutic effects in breast cancer

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The WHO has reported breast cancer (BC) to be the most frequent cancer in women. Currently, tamoxifen (TAM) which is mainly administered orally and via the parenteral route has limited aqueous solubility. Although oral administration is quite effective, TAM exhibits other infrequent side effects and acquired drug resistance on long-term therapy. Hence, the need for alternative modes of treatment which will improve the drug bioavailability, and be cost-effective, thereby enhancing the quality of life of BC patients and consequently, improving patient compliance and disease prognosis. Transdermal drug delivery offers numerous advantages over other drug delivery routes, including non-invasiveness, and the potential for continuous/controlled delivery. The major obstacle of the transdermal delivery route is the heterogeneous nature of the human skin barrier which leads to a considerable problem

in the delivery. The aim of our study was to develop a prototype of Nanoemulsion formulations containing curcumin as a natural bioactive compound which is a known anti-cancer agent.
Methods: Nanoemulsions were prepared using various oils and tested as carriers that improve permeation across the skin. The therapeutic effects of these formulations were evaluated using a syngeneic mouse model of BC. Transdermal delivery was used to achieve a targeted and localised drug delivery, as this can overcome the limitations of oral bioavailability in natural bioactive compounds with anti-cancer properties. At autopsy, the breast tumour, lungs, and liver were harvested from all the animals for histopathological and gene expression analysis.
Results: The modified Bloom Richardson grading system was used to grade the breast tumour excised from the vehicle and curcumin groups. A higher percentage of necrosis (N) was observed in tumour sections taken from mice fed with curcumin (25-70%) when compared with animals fed with vehicle (10-50%); or applied with curcumin-NE (10-40%). Immunohistochemical analysis of the breast tumours showed negativity for ER-alpha. The expression of CD9, TIMP-1, and MMP-13 showed a higher immunoreactivity score in the Curcumin Nanoemulsion(CNE), compared to the vehicle-fed group. Gene expression analysis of the four-candidate genes (CDH1/E-cadherin, TWIST1, API5 and CD274/PD-L1) showed that the oral administration and the nanoemulsion application were able to downregulate these genes while the nanoemulsion more significantly inhibited these genes compared to oral administration.
Conclusion: Nanoemulsion-based delivery has better efficacy against aggressive mouse breast tumours when compared with oral administration. This will be useful in designing newer modes of delivery for bioactive compounds and new therapies for the treatment of BC.

Keywords: breast cancer, nanoemulsion, curcumin, topical application, mouse model

P18-1

Enhancing physiology learning and practical experimentation with the combination of task-based modul and powerlab in undergraduate medical students

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Background: Anatomy, physiology, and biochemistry were known as basic sciences that are crucial in practicing medicine. Task-based learning was another approach that could be used in teaching basic sciences. In task-based learning, students were asked to complete tasks. Teaching in physiology, particularly in undergraduate medical students involving laboratory exercises and experiments, has evolved significantly due to the introduction of computer-aided data acquisition (DAQ) systems. The aim of this study is to evaluate how the combination of task-based modules and Powerlab enhances physiology learning in undergraduate medical students.
Methods: This study was conducted among 180 undergraduate medical students in Faculty of Medicine and Health sciences, Unismuh. A questionnaire was used to investigate their perceptions of the use of Powerlab in conducting and teaching physiology experiments. The task-based module was also used. Student's cognitive abilities were measured with a pre-posttest, practicum exam, and final mark of the biomedical course. The data were analyzed using SPSS and Microsoft excel. Frequency and summary statistics were analyzed using descriptive analysis.
Results: There was 74.4% of students showed a good to excellent learning and understanding of physiology concepts during experiments with Powerlab. The pre-posttest score elicited a statistically significant median increase compared to the pre-test score (p-value <0.005). There was also a statistically significant correlation between practicum exam results and average test scores with the final mark in the Biomedical course with a p-value <0.05.
Conclusion: The application of computer-based data using Powerlab and a combination of Task-based modules were proven to correlate with students' grades in Biomedical courses. These methods could provide students with hands-on and early exposure to concepts in physiology.

Keywords: Powerlab, physiology, medical students, learning

P18-2

The role of student standardized patients use in PBL teaching in the construction of first-class courses in physiology

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Background: In order to promote the construction of first-class courses in physiology, we apply student standardized patients (SSP) in PBL teaching and strengthen the ideological and political construction of the course.
Methods: In this study, four PBL groups in a class of clinical medicine specialty were selected for comparison, two of which were control group and the other two were experimental group. The experimental group adopted PBL combined with student standardized patient teaching method, and integrated ideological and political elements into the curriculum. The teaching effect of the two groups was observed through student self-evaluation, student mutual evaluation, teacher evaluation and basic theoretical knowledge examination.
Results: There was no significant difference between the experimental group and the control group for the PBL students' self-assessment and theoretical knowledge test scores(P>0.05), student mutual evaluation and teacher evaluation results of the experimental group were better than those of the control group, and the difference was statistically significant (P<0.05).
Conclusion: In this study, student standardized patients were used in PBL teaching, and the teaching effect was better than that of traditional PBL teaching. The integration of ideological and political education into the curriculum can promote students' improvement of professional spirit and literacy.

Keywords: student standardized patients, physiology, PBL teaching, teaching effect, education

Poster

P18-3

Development of board games intervention for students to enhance the knowledge on depression, anxiety and stress management

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Board games have been demonstrated to improve adults with cognitive impairment as well as children and adults with illness-prone behaviours. Board games also encourage youngsters to learn how to adhere to rules and to sit for extended periods of time, which can improve their concentration. According to World Health Organization (WHO), over 300 million people worldwide are believed to suffer from depression, accounting for 4.4 percent of the global population. The number of people suffering from common mental diseases is rising worldwide, particularly in low-income nations, as the population grows and more people reach the age when depression and anxiety are most common. Their knowledge about its causes was inaccurate, lacking of understanding about depression, anxiety and stress and its healing makes this mental health issue remain unnoticed and untreated where they do not seek for any treatment. Therefore, introducing depression, anxiety and stress education through interactive board games gain more benefits together enhanced the knowledge and attitude level towards depression, anxiety and stress. This was an intervention study focusing on formulating and developing interactive board games as an interventional approach to improve knowledge and attitude level on depression, anxiety and stress. Feasibility and acceptability of the interactive board games has face validate by respondents and content validate by experts. Three interactive board games with five modules of each developed named as (DAS EduKit- D(Depression), DAS EduKit – A (Anxiety), DAS EduKit- S (Stress)) which consisted of module 1 sign and symptoms, module 2 types of depression, anxiety & stress, module 3 causes, module 4 risk factors, module 5 way to cope. Respondents and experts commented the interactive board games really beneficial for the students to learn and gain knowledge about depression, anxiety and stress. DAS EduKit- D, DAS EduKit – A, DAS EduKit- S gained positive feedback from the assessment. Newly develop DAS EduKit- D, DAS EduKit – A, DAS EduKit- S were feasible and acceptable among students. This interactive board games can be alternative and interactive game for better understanding on depression, anxiety and stress management among students.

Keywords: depression, anxiety, stress, knowledge, interactive board games

P18-4

Physiology quiz in Japan: an international quiz-style online competition as the novel medical education for the next generation

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Background: Physiology Quiz in Japan (PQJ) has been held annually since 2016 as an international quiz competition on basic medicine, including physiology, hosted by Japanese university students. While learning basic medicine is important, there are few opportunities to compare one's level of achievement in basic medical knowledge with that of other students. Furthermore, the recent outbreak of coronavirus infection (COVID-19) has reduced opportunities for students to interact with each other. Here, we reviewed the outcomes of the online event PQJ 2023, held by students at Sapporo Medical University on March 26, 2023.

Methods: Participants must be university students, regardless of academic years and nationality. A team consisted of 2-5 members from the same university. The competition was conducted online via Zoom, with participants discussing within their teams and submitting one response to each question. The online tool AhaSlides was used for presention, answering, and scoring in the first and second rounds. All teams participated in the first round, and the top 30 teams advanced to the second round. A total of 8 teams, including one team that passed the consolation round, advanced to the final round. The team with the highest rate of correct answers was the champion. The questions were originally conceived by the students and were supervised by the faculties of the Department of Physiology at Sapporo Medical University. The members of the Education Committee of the Physiological Society of Japan served as judges. The program was conducted entirely in English.

Results: Nearly 500 students in 125 teams from 12 countries and regions, including Belgium, China, Czech Republic, Egypt, Indonesia, Japan, Macau, Malaysia, Mongolia, Peru, Slovenia, and Vietnam, participated in PQJ 2023, the largest number of teams in the history of PQJ. Both the Japanese and Slovenian teams won this competition. In terms of difficulty, the four-choice question with the highest rate of correct answers in the first round was the question about ions required for glucose absorption in the small intestine (72.6% correct), while the question with the lowest rate of correct answers was the question about the organ with the most reduced blood flow when blood vessels are not elastic (24.2% correct).

Conclusion: The successful closing of PQJ 2023, with the largest number of teams ever, clearly indicates the growing demand for the quiz-based medical education using online tools. PQJ may be not only an opportunity for testing medical knowledge but also an excellent platform to develop international communication skills and friendships for the next generation regardless of the COVID-19 pandemic.

Keywords: PQJ, education, online, quiz, international

P18-5

Satisfaction from academic activities among final year students in the University of Medicine, Magway, Myanmar

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Background: Nowadays, medical education is transforming from the teacher-centered to the student-centered approach. Since students are at the center of the education process, student satisfaction is equally important to customer satisfaction. Student satisfaction is related to several outcome variables, such as persistence, retention, course quality, and student success. The satisfaction of students with learning/teaching methods, learning/teaching environments and assessment methods should be evaluated for the improvement of an educational program. Therefore, this study aims to assess the satisfaction from academic activities among final year students in the University of Medicine, Magway.
Methodology: A cross-sectional descriptive study was conducted on 63 final part II senior students who had completed at least two rounds of clinical rotation. The mixed-method was employed, utilizing both a questionnaire and interviews. The students were asked to complete online questionnaires to assess their satisfaction from the traditional discipline-based curriculum. Additionally, eight students were individually interviewed via the Zoom application to gather more detailed information.
Results: The 54% of students were satisfied with the learning materials and methods. The 66.7% of students thought that the assessment system was fair. More than 50% of students were satisfied with the workspace for study. Two-thirds of students felt that the 2nd year was rigorous, while half of the students believed that the final year was rigorous for them.
Conclusion: Student satisfaction level is good for course delivery, faculties and assessment. However, most of the students believed that the current academic program was outdated and they wanted to upgrade the program. They also wanted better in-campus facilities, such as a bookstore, computer and internet facilities, and a counseling center.

Keywords: student satisfaction, academic activities, final year medical students, traditional discipline-based curriculum

P18-6

From integrated teaching team to integrated course - problem-based learning was adopted in Hainan Medical University

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Background: To solve the problem of having a shortage of tutors in comparison to a large number of students in problem-based learning for promoting students' abilities and cultivating excellent medical talents.
Methods: Recruiting 45 teachers from 11 different departments to build a teaching team for problem-based learning (PBL) in the Hainan Medical University. An integrated course of PBL was created by the teaching team with 8 clinical cases which involved multiple subjects and different systems. Each case study was designed in three sessions and occupied 6 class hours in 10 days, and the course covered two semesters. There were 560~614 medical students who received PBL each year from 2018 to 2023.The students were divided into 53 groups, with 10 to 12 students in each group. Formative assessment, summative assessment, and questionnaire survey were used to evaluate the effects of the integrated course of PBL.
Results: Students' knowledge, abilities and attitudes were developed significantly. For example, 95.0% of students developed the knowledge of basic and clinical medicine; 92.4% of students developed the abilities of discovering and solving problems; 86.0% of students developed scientific reasoning and critical thinking; 100.0% of students developed information literacy; 87.0% of students developed self-directed learning; 96.0% of students realized the importance of psycho-social issues, professional spirit, and social responsibility of becoming a doctor.
Conclusion: Creating an integrated teaching team can solve the problem of having a shortage of tutors in comparison to a large number of students in problem-based learning and an integrated problem-based learning course is important for medical education.

Keywords: problem-based learning, integrated PBL course, integrated teaching team, excellent medical talents

P18-7

Automated grading of medical students' learning outcome of physiological mechanisms using deep learning models

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Physiology is a compulsory subject for medical students before they enter clinical medicine. Physiology explores the responses of the body's organs to stimuli and their interactions with each other, so medical students must pay particular attention to the causal relationships between various physiological parameters to grasp the full range of physiological mechanisms. However, most medical faculties' physiology examinations and the first stage of the National Examination for Doctors administered by the Department of Examinations are predominantly single-choice. The disadvantage of relying exclusively on a multiple-choice question (MCQ) tests is that the content of the tests is mostly narrative and factual but does not assess the full range of concepts and overall comprehension. Conversely, if we were to switch to essay questions for various physiological assessments in order to rectify the drawbacks of relying on multiple-choice questions, there would be difficulties in terms of time spent on grading, lack of qualified evaluators, and doubts about fairness. To address these issues, we asked medical students to map physiological mechanisms into a flowchart and utilized an automated grading system to evaluate students' flowcharts. In this study, we have developed a system using two deep learning models, Long Short-Term Memory (LSTM) and Transformer network architecture, plus linear regression, to grade answers in the flowchart. The system was trained to learn the way of teacher's grading, then the medical students' responses were graded automatically and compared to the teacher's grading. The results of the study confirmed that the scores of the automated grading and the teacher's grading were very close to each other. We have achieved a mean squared error (MSE) lower than 3 when comparing the scores of teachers' grading with those of the models. Based on the findings of this study, there is potential for future changes in the way physiology learning outcomes are assessed to improve the effectiveness of medical students' learning of physiological mechanisms.

Keywords: physiological mechanism, learning outcome, automated grading, evaluation, deep learning model

Poster

P18-8

Competition—a method of promoting teaching: Brief introduction of physiological quiz for Chinese students in medical and health-related major

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Background: Physiological quiz for Chinese students in medical and health-related majors began in 2015 and are held annually, about in June or July. The quizzes were mainly hosted and undertaken by the Physiology department of Xuzhou Medical University, supported by the Organizing Committee of the international physiology quiz, the Chinese Physiological Society and the Jiangsu physiological science society. So far, seven sessions have been successfully held. 115 universities participated in the seventh session.
Methods and Contents: Each university sent one or two representative teams, each team consisting of 3-5 students. The Physiology quizzes includes the written test and the oral test. During the student quiz, teachers also specially held a physiology teaching seminar to give suggestions on how to improve students' interest in physiology learning. For example, the theme of last year's seminar was "The Application of Modern Information Technology in Physiology Teaching"; The theme of this year's discussion is "Discussion on Digital Teaching Forms of Physiology".
Significance: Our physiology quizzes that students find interesting and willing to participate in actively have become an effective measures of improving physiology teaching.

Keywords: physiological quiz, motivational teaching, teaching reform, medical and health-related majors, China

P19-1

Unveiling the potentiality of shikonin derivatives inhibiting SARS-CoV-2 main protease by molecular dynamic simulation studies

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Shikonin, a phytochemical present in the roots of *Lithospermum erythrorhizon*, is well-known for its broad-spectrum activity against cancer, oxidative stress, inflammation, viruses, and anti-COVID-19 agents. A recent report based on a crystallographic study revealed a distinct conformation of shikonin binding to the SARS-CoV-2 main protease (M^{pro}), suggesting the possibility of designing potential inhibitors based on shikonin derivatives. The present study aimed to identify potential shikonin derivatives targeting the M^{pro} of COVID-19 by using molecular docking and molecular dynamics simulations. A total of 20 shikonin derivatives were screened, of which few derivatives showed higher binding affinity than shikonin. Following the MM-GBSA binding energy calculations using the docked structures, four derivatives were retained with the highest binding energy and subjected to molecular dynamics simulation. Molecular dynamics simulation studies suggested that alpha-methyl-n-butyl shikonin, beta-hydroxyisovaleryl shikonin, and lithospermidin-B interacted with two conserved residues, His41 and Cys145, through multiple bonding in the catalytic sites. This suggests that these residues may effectively suppress SARS-CoV-2 progression by inhibiting M^{pro}. Taken together, the present in silico study concluded that shikonin derivatives may play an influential role in M^{pro} inhibition.

Keywords: SARS-CoV-2, main protease, shikonin derivatives, molecular docking, molecular dynamics simulation

P19-2

Identifying the multi-target pharmacological mechanism of action of genistein on lung cancer by integrating network pharmacology and molecular docking

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In lung cancer, many genetic abnormalities result in several oncogene activations and tumor-suppressor gene inactivation. Still, the need for in-depth molecular mechanisms limits their particular role and active modulators. Genistein, a natural isoflavone in soybeans, has gained potential interest as an anti-cancerous agent in various cancers. However, the effects of genistein on lung cancer still need to be clarified. With this regard, we used network pharmacology and *in-silico* approaches to unravel molecule mechanisms of genistein with its corresponding targets. Common targets relevant to genistein and lung cancer were verified through the UniPort database and subjected to STRING biological databases to obtain protein-protein interaction. Based on network pharmacology and degree score, genistein may significantly interact with the top 10 hub genes, including AKT1, EGFR, ESR1, STAT3, PRAG, MMP9, SRC, CASP3, PTGS2, and AR, which are closely related to cell survival, cell migration, cell permeability, and apoptosis following VEGF and Prolactin signaling pathway. Furthermore, molecular docking and dynamics simulation analysis confirmed genistein showed significant binding affinity to some of the critical targets, including AKT1, EGFR, and STAT3, which are essential regulators of molecular and cellular processes linked with non small cell lung cancer progression. Thus, the current system pharmacology and in silico data exhibited genistein might modulate lung cancer pathobiology significantly, suggesting its therapeutic applicability for preventing and treating lung cancer.

Keywords: genistein, lung cancer, AKT1, EGFR, molecular docking

P19-3

Structure-based identification of potent modulators from *Centella Asiatica* targeting BACE-1

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Centella Asiatica, commonly known as goto kola and a member of the Apiaceae family, has been demonstrated to be a beneficial medicinal plant with diverse pharmacological activities against various diseases, including memory enhancement, cognitive enhancement improvement, anticholinergic and antioxidative effects. Alzheimer's is the most common age-related neurodegenerative disease. Multiple research studies have linked BACE-1 inhibitors to a reduction in Alzheimer's disease pathology, and the medication has shown to be highly successful. The present study aimed to identify potential BACE-1 inhibitors by using ADMET analysis for chemical profiling, and molecular docking and

molecular dynamic simulation were used for potent inhibitor selection. ADMET prediction showed that approximately 63.63% of compounds showed the drug-likeness property and crossed the blood-brain barrier. Three compounds have been screened out by molecular docking and MMGBSA, wherein Myricetin,Quercetin and cadiyenol showed better binding energy. Moreover, molecular dynamic simulation studies suggested that Myricetin interacted with the catalytic dyad of BACE-1. Overall, the present in silico study suggested that Centella Asiatica isolated compounds as a significant source of a BACE-1 inhibitor for AD treatments.

Keywords: Alzheimer Disease, BACE-1, centella asiatica, molecular docking, molecular dynamic simulation

P19-4

Effects of chitooligosaccharide-epigallocatechin gallate conjugates on neurodegeneration in rats fed a high-fat diet

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Obesity is a serious metabolic problem worldwide caused by high-fat diet (HFD) consumption. The long-term consumption of a HFD contributes to the pathogenesis of neurodegeneration, which is responsible for impairment of cognitive functions. It has been reported that HFD causes high levels of phosphorylated Tau (p-Tau) proteins in the brain tissues. The successful grafting of chitooligosaccharide (COS) with epigallocatechin gallate (EGCG), acts as a neuroprotective effect. Nowadays, the effect of COS-EGCG on HFD-induced brain pathology is still unclear. Therefore, this study aimed to investigate the effects of COS-EGCG to improve cognitive impairment in HFD rats. Twenty-four male Wistar rats were divided into normal diet control group (ND, n = 6) (fat representing 19.79% of total calories), and high-fat diet control group (HFD, n = 18) (fat representing 65.26% of total calories) for 20 weeks. At 16 weeks after HFD-induced rats, the obesity was identified successfully in HFD rats then received treatment for 4 weeks. The rats were divided into 4 groups: ND, HFD (HFD), HFD with COS-EGCG at dose 600 mg/kg body weight (COS-EGCG), and HFD with Atorvastatin (Ator) as positive control. Moreover, one week before being sacrificed, the rats of each group were assessed the cognitive function by using the Morris water maze (MWM) test. At 20 weeks, the rats were sacrificed and removed from the brain to study the histopathological alteration by hematoxylin and eosin (H&E) staining. In addition, the hippocampus was examined for the expression of p-Tau proteins by Western blot analysis. The results of the MWM test showed that there was reduced escape latency time, and escape latency time at day 5, but increased time in target quadrant at day 6, and platform crossing in the COS-EGCG treatment in rats compared to the HFD rats. In H&E sections, COS-EGCG showed the morphology of neurons close to ND rats that had a pyramidal shape with clear cytoplasm, and a single nucleus and prominent nucleolus in the CA1 region of the hippocampus. Whereas, HFD rats were observed to have some degenerated neurons which is an irregular shape of neuron with darkly basophilic-stained cytoplasm and nucleus. Moreover, the formation of vacuole was observed in its cytoplasm, compared to ND rats. Treatment with COS-EGCG reduced neurodegeneration by a measurable decrease in p-Tau proteins in the hippocampus. This study suggested that COS-EGCG had a neuroprotective effect on HFD-induced obesity by diminishing neurodegeneration and might restore spatial learning and long-term memory ability in rats.

Keywords: chitooligosaccharides-epigallocatechin gallate, obesity, high-fat diet, Alzheimer's disease, neurodegeneration

P19-5

The effect of chitooligosaccharides conjugated with epigallocatechin gallate on lipid accumulation in obese rats

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Overweight and obesity have become a worldwide health problem, caused by excessive fat accumulation in adipose tissue. In this study, we investigated the anti-obesity effects on chitooligosaccharides (COS) conjugated with epigallocatechin gallate (EGCG) in obese rats. Male Wistar rats were fed a high-fat diet for 16 weeks to induce obesity. Rats were randomly divided into normal diet group and high fat diet (HFD) group, rats with HFD groups were subdivided into 3 groups: HFD group, HFD + COS conjugated EGCG (600 mg/kg/day) group, HFD + Ator (10 mg/kg/day) group for additional 4 weeks. At the end of this study, the rats were measured BW and sacrificed to remove adipose tissue and collected at -80C°. Our results found that the body weight and white adipose tissue weight in HFD + COS-EGCG group were significantly reduced when compared with the HFD group. Moreover, the HFD + COS-EGCG group also improved serum lipid profiles and reduced lipid accumulation as demonstrated by attenuated adipocyte size and triglyceride content in white adipose tissue. All these results suggest that COS-EGCG has beneficial effects on lipid accumulation; it might be a novel strategy to prevent obesity and other metabolic disease.

Keywords: chitooligosaccharides, epigallocatechin gallate, white adipose tissue, high-fat diet, obesity

P19-6

The effects of chitooligosaccharides-epigallocatechin gallate conjugates on obesity-associated metabolic disorders in high-fat diet rats

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High consumption of fat has been linked to obesity-associated metabolic disorders. Chitooligosaccharide (COS) and epigallocatechin gallate (EGCG) have been reported to have potential anti-obesity effects. Several studies of conjugated compounds showed higher activities than its pure compound. Therefore, the aims of this study were to investigate the therapeutic effects of COS-EGCG conjugates on high-fat diet (HFD)

in rats and evaluated its beneficial mechanisms involved in obesity-associated metabolic disorders. The male Wistar rats were divided into 4 groups including normal diet (ND), HFD, HFD received COS-EGCG conjugates (HFD+CE), and HFD received Atorvastatin (HFD+ATV). The rats were fed a ND or HFD for 20 weeks. After 16 weeks, the rats received the treatment for 4 weeks. The results showed that COS-EGCG conjugates decreased metabolic parameters compared to HFD rats. Furthermore, COS-EGCG conjugates could reduce parameters that related to hepatic lipid accumulation and hepatic injury such as hepatic triglyceride (TG), hepatic total cholesterol (TC), serum Aspartate Transaminase (AST), and serum alanine aminotransferase (ALT). In conclusion, this study suggests that COS-EGCG conjugates could alleviate obesity, which is induced by HFD consumption as well as metabolic comorbidities such as non-alcoholic fatty liver disease (NAFLD).

Keywords: chitooligosaccharide, epigallocatechin gallate, high-fat diet, metabolic disorders, non-alcoholic fatty liver disease

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P19-7

The downregulation of ANO1 by Schisandrathera D presents a promising therapeutic target for the management of prostate and oral cancers

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Anoctamin 1 (ANO1) is an intracellular calcium-activated chloride ion channel that plays multiple physiopathological functions, particularly in cancer growth and metastasis induction. This study evaluated schisandrathera D, a novel compound isolated from Schisandra sphenanthera, for its effect on ANO1. Schisandrathera D inhibited the ANO1 activation-mediated decrease in yellow fluorescent protein fluorescence in a dose-dependent manner; however, it did not affect the adenosine triphosphate-induced increase in intracellular calcium concentration or the forskolin-induced cystic fibrosis transmembrane conductance regulator activity. Schisandrathera D was observed to cause a gradual decline in ANO1 protein levels and a notable decrease in cell viability in ANO1-expressing cells compared to ANO1-knockout cells. The effects observed may be due to the superior binding capability of Schisandrathera D towards ANO1 protein compared to Ani9, a previously identified ANO1 inhibitor. The investigation discovered the administration of schisandrathera D caused an increase in caspase-3 and cleaved poly (ADP-ribose) polymerase 1 levels, suggesting that its anti-cancer properties are attributed to the induction of apoptosis. The present study emphasizes the potential of schisandrathera D as an anticancer agent for prostate and oral cancers because of being ability to reduce ANO1 protein levels and induce apoptosis-mediated anticancer effects. Further research in this direction may lead the way for the development of novel therapeutic interventions.

Keywords: Ano1, Schisandrathera D, prostate cancer, oral cancer, molecular docking

P19-8

Acupuncture stimulation promoted anti-inflammation signaling in the medial septum and restored caffein induced hyperarousal in the rats

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Acupuncture is used to control various emotional disorders. HT7 stimulation has been found to improve insomnia and depression, and to modulate inflammatory cytokines. The study's objective was to evaluate the potential of HT7 for modulating the inflammatory response in the medial septum (MS) region in rats in which insomnia was induced by administration of caffeine for 2 weeks. Rats were randomly assigned to 4 groups: control (DW), caffeine (Caff), caffeine + acupuncture (HT7), and caffeine + nonacupuncture (NA). Wake, REM, and nREM sleep durations were evaluated using a rat wireless EEG measurement device. The behavioral patterns of rats were measured through an open field test. Anti- and pro-inflammatory cytokine levels were measured in the MS region using a cytokine array. The levels of M2 microglia (arginase-1) in the MS region were confirmed through immunofluorescence staining. IL-10 and IL-4 levels were measured by ELISA. Finally, the expression levels of pSTAT6/STAT6 and p-P38/P38 were estimated by Western blot assay.HT7 stimulation restored the arousal time increased by caffeine. HT7 stimulation increased M2 microglia expression and increased anti-inflammatory cytokine levels in the MS region. In addition, increased expression of IL-4 regulated the phosphorylation of STAT6 and P38, which are members of sub signaling pathways. HT7 stimulation may improve sleep patterns by regulating the cholinergic anti-inflammatory pathway and increasing the expression of anti-inflammatory markers.

Keywords: caffeine, acupuncuture, HT7, STAT6, P38

P19-9

Nutraceutical effects of *Hibiscus Sabdariffa* linn on cardiac energy metabolism and aortic tissue oxidative levels in rats fed with vitamin B12 restricted diet

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Noncommunicable diseases (NCDs), such as cardiovascular disease, account for 70% of global fatalities. The majority of cardiovascular diseases, including coronary heart disease, ischemic necrosis, aortic aneurysm, heart failure, and strokes, are caused by atherosclerosis. Endothelial dysfunction may be a cause or a precursor of the inflammatory and atherogenic processes. A deficiency in vitamin B12 can lead to endothelial dysfunction through the mechanism of oxidative stress, which is activated by elevated homocysteine levels in the blood. The scientific evidence for the assertion that plants and their active constituents play a crucial role in preventing chronic and degenerative diseases is expanding continuously. The purpose of the present study was to determine the protective effect of Hibiscus sabdariffa Linn. dried calyx ethanolic extract (HSE) on the plasma lipid profile, cardiac protein level, oxidative stress, histopathological characteristics of aortic tissue, and vitamin B12 status of Sprague Dawley rats with vitamin B12 deficiency. The treatment groups consisted of rats fed a vitamin B12-restricted diet for 8 or 16 weeks in conjunction with 400 mg/KgBW HSE. Positive con-

trol rats were fed a vitamin B12-restricted diet without HSE. Negative control rats were fed a control feed diet without HSE. The standard diet used was AIN-93M, whereas the vitamin B12 restriction diet was modified AIN-93M plus 5% pectin. The levels of total cholesterol, triglycerides, vitamin B12, homocysteine, PGC1-, and CPT1B were determined by sandwich enzyme-linked immunosorbent assay (ELISA). Healthy aortic tissue was utilized to measure SOD (colorimetry), MDA (spectrophotometry) and to obtain HE stains (histopathology). Data were analyzed with ANOVA using SPSS software. After 16 weeks of HSE treatment, total cholesterol and triglyceride levels were significantly lower ($p<0.001$), whereas cardiac cobalamin levels were significantly higher ($p<0.05$). After 8 and 16 weeks of HSE treatment, total homocysteine levels in cardiac tissue were significantly lower ($p<0.05$), whereas PGC1- and CPT1B levels in cardiac tissue were higher, although not significant. MDA levels and tunica intima/media ratio were significantly lower after 8 and 16 weeks of HSE treatment ($p<0.05$). In addition, vitamin B12-deficient rats treated with HSE demonstrated an increase in SOD enzyme activity. We concluded that HSE administration prevented vitamin B12 deficiency in rats fed a vitamin B12-restricted diet, while simultaneously enhancing the lipid profile and cardiac energy metabolism. and also diminish oxidative stress.

Keywords: Hibiscus sabdariffa Linn, Vit B12 deficiency, cardiac protein, oxidative stress

P19-10

Effect of moringa oleifera leaf extract on FGF21 MRNA expression in male wistar rats skeletal muscle

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Background: FGF21 is one of skeletal muscle's myokines whose expression may be influenced by an increased ROS. Several conditions may alter ROS level in the body including antioxidant. Moringa oleifera, known as Miracle Tree, contains numerous pharmacological effects including antioxidant substances. Considering a potent antioxidant in this plant, Moringa oleifera is potential to modify FGF21 expression in skeletal muscles as it may interfere the oxidative stress pathway. This study aims to identify the effect of Moringa oleifera leaf extract towards FGF21 mRNA expression and mitochondria oxidative function in skeletal muscle.
Methods: We studied twelve sedentary rats divided into two groups, control and treatment, with the treatment group given 200mg/kg Moringa oleifera leaf extract for 12 weeks. RT-PCR method was done to analyze COX IV and FGF21 mRNA in soleus muscle.
Results: Results showed lower COX IV level (P=0.354) in contrast with the higher expression of FGF21 in the treatment group given Moringa oleifera leaf extract (P=0.170).
Conclusion: The exact impact of Moringa oleifera to skeletal muscle FGF21 is still unclear. However, our study provides new insights that FGF21 correlates with mitochondrial content, activity and biogenesis which act as a novel therapy response towards the decline of mitochondrial oxidative and respiratory capacity.

Keywords: Moringa oleifera, skeletal muscle, FGF21, COX IV, mitochondria

P19-11

Efficacy analysis of fermented *Cordyceps cicadae* for amelioration of cataract formation

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Cataract is one of the major causes of vision impairment and blindness. The most prevalent risk factors are ageing, genetics, and post-surgery damages. The symptoms include fuzziness, photophobia, and reduced color sensitivity. The ancient Chinese medicine literature had referred Cordyceps cicadae as having effects for vision enhancement and antipterygium, but in lack of experimental evidence to support such claim. Thus, this study investigated 5 Cordyceps cicadae products, either dissolved in 0.9% NaCl (sample B and E) or soy bean oil (sample A, C, and D), in 5 to 6 weeks-old ICR female mice for their preventive effects against cataract formation induced by UVB (Ultraviolet B) irradiation. The study lasted for 28 days and included 9 study groups: (1)Saline blank group (fed with 0.2 ml 0.9% saline), (2) Saline UVB group (fed with 0.2 ml 0.9% saline + UVB irradiation), (3)Sample B (fed with 0.2 ml sample B + UVB irradiation), (4) Sample E (fed with 0.2 ml sample E + UVB irradiation), (5) Soy bean oil blank (fed with 0.2 ml soy bean oil), (6) Soy bean oil UVB group (fed with 0.2 ml soybean oil + UVB irradiation), (7) Sample A (fed with 0.2 ml sample A + UVB irradiation), (8) Sample C (fed with 0.2 ml sample C + UVB irradiation), (9) Sample D (fed with 0.2 ml sample D + UVB irradiation). All samples are at 100 mg/Kg dosage. The schedule was set as Day1 ~ Day28 for tube-feeding, Day6 ~ Day28 for UVB irradiation, and Day29 for sacrifice. Slip-lamp like scanning on lens, post-lens image, and lens light transmission analysis were performed before sacrifice, followed by tissue sectioning and immunohistochemical staining for mechanism analyses. The results showed that the samples A, B, and E were beneficial for cataract prevention. The underlying mechanisms have been partly unveiled. This study supports the potential of *Cordyceps cicadae* fermented products for development as an anti-cataract formation functional food.

Keywords: cataract, Cordyceps cicadae fermented products, prevention, functional food

P19-12

Vasorelaxant properties of hydrolyzed collagen from salmon skin on isolated rat aorta

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Hydrolyzed collagen (HC) comprised of small peptides by low molecular weight (3-6 KDa). HC is known to exert antioxidant, antimicrobial and antihypertensive capacity. However, the mechanism by which HC regulates vasorelaxation remains unclear. Therefore, we aimed to inves-

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tigate the effects of HC related to antihypertensive effect on mechanisms. Vascular reactivity experiments were performed in isolated rat thoracic aortic rings using an organ bath system. The aortic rings were pre-contracted by PE or KCl to verify the endothelium-dependent pathways, and to investigate with K^+ channels and α_1-adrenergic receptor mechanisms for vascular relaxation induced by HC.
HC inhibited the rat aortic ring contraction evolked by phenylephrine and KCl in a concentration-dependent manner. HC showed an inhibitory effect on vasocontraction of aorta with high concentration of potassium. K^+ channels and α_1-adrenergic receptor mechanisms show that hydrolyzed collagen operated through smooth muscle cell to vasorelaxation.
The data concerning the benefits of HC might be further investigated for the application of HC as an antihypertensive compound.

Keywords: hydrolyzed collagen, rat aortic ring, vascular relaxation, endothelium, hypertension

Poster

P19-13

Genistein attenuates diet-induced obesity and metabolic dysfunctions in gonadectomized mice with some sex-differential features

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The prevalence of obesity and its associated metabolic dysfunctions, such as insulin resistance (IR) and metabolic dysfunction-associated steatotic liver disease (MASLD), is increasing worldwide and closely linked to aging. Sex hormones, particularly estrogens, considerably contribute to the sex differences in susceptibility to metabolic dysfunctions. This is evident from the increased prevalence of obesity, IR, and diabetes in women after menopause. Genistein, an isoflavone (phytoestrogen) extracted from legumes, has been identified as a potential natural compound for the prevention and treatment of obesity and metabolic dysfunctions. However, it remains to be investigated whether genistein exhibits sex-specific protective effects on obesity, IR, and MASLD. Therefore, we studied the effect of genistein on metabolic dysfunctions in gonadectomized male and female mice upon high-fat high-sucrose diet (HFD)-induced obesity.
Fourteen male and fourteen female C57BL/6 mice were gonadectomized at 8 weeks of age. One week after gonadectomy, the mice were given ad libitum access to the HFD (30% lard and 9% sucrose). Starting at 13 weeks of age, genistein (16 mg/kg) or vehicle (7.5% DMSO) was administered orally, five days a week, for 8 weeks (n=7/group). Then, an intraperitoneal glucose tolerance test (IPGTT) was performed. One week later, serum and tissues were collected for biochemical, histopathological, and molecular analyses.
Compared to vehicle-treated mice, genistein treatment sex-independently resulted in a 15.1±10.8% reduction in body weight ($p<0.01$), a 17.6±9.8% reduction in the liver index (ratio of liver to body weight, $p<0.01$), a 42.4±26.9% reduction in serum ALT levels, and a 52.3±32.1% reduction in hepatic steatosis score (blindly assessed by a pathologist, $p<0.01$). Genistein treatment also attenuated HFD-induced IR, as demonstrated by a 21.8±32.1% improvement in IPGTT (calculated by the AUC, $p<0.01$) and a 64.3±38.5% reduction in the homeostasis model assessment of insulin resistance (HOMA-IR) index ($p=0.01$). Regarding hepatic gene expression, genistein treatment reduced the mRNA expression of the lipogenic enzyme *Fasn*, its transcription factor *Srebf1*, and MASLD progression markers such as *Cd36*, *Col1a1*, and *Saa1*. Furthermore, the downregulation of these genes by genistein treatment was more pronounced in female mice than in male mice.
In conclusion, genistein treatment alleviated HFD-induced obesity, IR, and MASLD in gonadectomized mice in various aspects, exhibiting sex-specific effects at the gene expression level in the liver. Thus, genistein may be a promising natural-derived therapy for metabolic dysfunctions, particularly in postmenopausal women. However, further studies are needed to determine whether genistein provides benefits to other metabolic tissues, such as skeletal muscles and adipose tissues.

Keywords: genistein, obesity, insulin resistance, metabolic dysfunction-associated steatotic liver disease (MASLD), sex difference

P19-14

Kelulut honey improves oestrus cycle, hormonal profiles, and oxidative stress markers in Letrozole-induced polycystic ovary syndrome rats

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Polycystic ovary syndrome (PCOS) is a complex disorder affecting the reproductive, endocrine, metabolic and psychological systems. Treatment for patients with PCOS focuses primarily on managing the associated symptoms, as there is no definitive cure for this condition. However, these medications are associated with several adverse effects, including diarrhoea, vaginal and uterine bleeding, breast tenderness, hot flushes and abdominal discomfort. Therefore, it is of great interest to find a natural supplement that can be used as a complementary treatment for PCOS and has minimal side effects. Kelulut honey (KH) is a multiflorous stingless bee honey from Trigona spp. that has been shown to have excellent antioxidant and anti-inflammatory properties with unique physicochemical properties. This study, therefore, investigated the isolated and combined effects of KH, metformin or clomiphene on alleviating oxidative stress, reproductive and metabolic abnormalities in PCOS. PCOS was induced in female Sprague-Dawley (SD) rats with 1 mg/kg/day letrozole for 21 days, excluding the normal control group. The PCOS rats were then divided into six treatment groups (n=6): untreated, metformin (500 mg/kg/day), clomiphene (2 mg/kg/day), KH (1 g/kg/day), combined KH (1 g/kg/day) and metformin (500 mg/kg/day) and combined KH (1 g/kg/day) and clomiphene (2 mg/kg/day). All treatments were administered orally for 35 days. This study showed that compared to untreated PCOS rats, KH significantly decreased the percentage of dioestrus days, serum testosterone and luteinising hormone levels, and decreased ovarian catalase activity, while total SOD and GSH activity increased. However, KH did not reduce fasting blood glucose, insulin and body weight gain in PCOS rats. These findings could thus provide a basis for future research on the possible use of KH as a complementary therapy for women with PCOS.

Keywords: kelulut honey, antioxidative, PCOS, complementary therapy

P19-15

Protective effect of lotus seed (Nelumbo nucifera) extract on male reproductive toxicity in L-NAME-induced hypertensive rats

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Background: *Nelumbo nucifera* (Lotus) seed is a medicinal plant traditionally used in tropical areas for the treatment of hypertension. It's also reported to possess anti-inflammatory and antioxidant effects. However, the effect of lotus seed on reproductive dysfunction associated with hypertension has never been elucidated. Therefore, this study investigated the preventive effects of lotus seed extract (LSE) on blood pressure and male reproductive function in Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME)-induced hypertensive rats.
Methods: Male Sprague–Dawley rats were randomly divided into four groups (n = 6): normotensive control rats; hypertensive rats (L-NAME alone 40 mg/kg/day); hypertensive rats treated with either LSE (10 mg/kg/day) or captopril (5 mg/kg/day) for 5 weeks. Blood pressure, sperm functional parameters, and serum testosterone were measured. Oxidative stress indices, antioxidant enzymes activities and histopathological alterations in the testes and epididymis were determined.
Results: The rats that received L-NAME exhibited high blood pressure and male reproductive toxicity via increased oxidative stress in testicular and epididymal tissues, decreased testosterone and sperm production, and more sperm abnormalities, potentially due to apoptosis. LSE significantly reduced blood pressure, increased concentrations of testosterone, the number of sperms and their progressive motility in the hypertensive rats. Moreover, administration of LSE markedly increased antioxidant enzymes activities, suppressed the increase in biomarkers of oxidative stress and improved testicular histology in the testes and epididymis of the hypertensive rats.
Conclusion: LSE can ameliorate reproductive toxicity in male rats with L-NAME-induced hypertension. The ability of LSE to restore male sex hormonal balance and spermatogenic function in hypertensive rats was attributed to its suppression of oxidative stress and enhancement of antioxidant defense mechanisms.

Keywords: Nelumbo nucifera, Lotus seed, reproductive dysfunction, hypertension, L-NAME

P19-16

Chrysosplenol C decreases mitochondrial reactive oxygen species and prevents pathologic progress to heart failure in transverse aortic constriction rat model

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Chrysosplenol C (4',5,6-trihydroxy-3,3',7-trimethoxyflavone, CPC) is a flavone contained in several medicinal plants including *Miliusa balansae*. This chemical increases cardiac myocyte contractility via protein kinase C (PKC)-dependent enhancements in Ca^{2+}-induced Ca^{2+} release and in Ca^{2+} sensitivity of ryanodine receptors (RyRs). We investigated if this chemical rescues the pathogenic progress from hypertrophy to heart failure (HF) using transverse aortic constriction (TAC) rat model, and underlying mechanism for CPC-induced beneficial effects on ventricular muscle. When the rats showing significant hypertrophy at 5-week TAC, were injected with CPC (intraperitoneal, 5 mg/kg/day) from 8-week TAC for further 8 weeks, they showed well-maintained ejection fraction (EF) and left ventricular (LV) wall thickness. In contrast, control group with DMSO injection showed pathologic decreases of EF and LV wall thickness. One of mechanisms for sensitization of RyR to Ca^{2+} is its oxidation. When we examined if CPC affects mitochondrial reactive oxygen species (miROS) with the fluorescence dye Mito-SOX using confocal imaging, we found that this chemical rather reduces the level of miROS in a concentration-dependent manner (EC_{50} of ~35 μM) similar to well-known miROS scavenger mito-TEMPO. Such CPC's miROS lowering effect was maintained and somewhat enhanced when cellular superoxide dismutase was inhibited. We further tested if miROS lowering effect by CPC is linked to PKC, and found that in the cells preincubated with PKC inhibitors, GF109203X or chelerythrine, CPC still decreased miROS to the similar extents. It was noted that GF109203X, but not chelerythrine, significantly increased miROS by itself. Our data suggests that CPC may prevent HF development by reduction of miROS and by enhancement of contractility under prolonged pressure overload.

Keywords: ventricular muscle, chrysosplenol C, mitochondrial ROS, heart failure, transverse aortic constriction

P19-17

Effects of thermotherapy on Irisin and orexin levels metabolic of factors in middle aged obese woman

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Many women gaining weight as they transition as they approach menopause. Weight gain during menopause is predominantly due to a reduction in physical activity. For women who are obesity menopause women, appropriate therapy about controlling weight and increasing lipid metabolism is required to prevent metabolic syndrome. The main aim of this study was to analyze the how thermotherapy (half bath in hot water, 42±0.5°C, 3-4 times/week, 30 min/time, 15 times for 4 weeks) affects the orexin A (OxA), adiponectin and c-reactive protein (CRP) expression in menopausal overweight-obese women(n=20, age, 56.17±3.83 yrs; height, 158.62±4.61 cm; weight, 65.47±6.28 kg). Significant increased of OxA ($p<0.01$), adiponectin ($p<0.05$) and CRP ($p<0.01$) after thermotherapy. We found that the increased lipid metabolism with thermotherapy was associated with the OxA and adiponectin. Also, the role of OxA on lifestyle and eating behavior in menopausal overweight-obese women can be further explored to identify obesity and lifestyle-related diseases.

Keywords: Thermotherapy, AOrexin, LAdiponectin, C-reactive protein

Poster

Poster

P19-18

Combinative nutraceutical affecting physiological responses lipidemia in vivo

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The problem of metabolic syndrome due to obesity, supported by sedentary activities, is still a problem. The use of nutraceuticals in society continues to grow, including efforts to combine efficacious ingredients to reduce body fat by controlling the physiological regulation of blood lipids. However, preclinical evidence of efficacy still needs to be improved—the research to explore combinative nutraceuticals/CN (red yeast rice, garlic, and red ginger) on the physiological effect of lipidemia levels in vivo. Forty-two male Wistar rats were divided into seven groups (Normal, HFD, HFD+Simvastatin (0.9 mg/kgBW), HFD+Dose 1 (54 mg/kgBW), HFD+Dose 2 (108 mg/kgBW), and HFD+Dose 3 (162 mg/kg BW). For the first seven days, acclimatization was carried out. Continued four weeks, the rats were induced dyslipidemia with HFD. After induction, the test treatment was continued for eight weeks. All rats from each group were taken as blood samples periodically (at H0 as a baseline, W4, and W8). All data was collected and analyzed statistically by ANOVA and post hoc Tukey by CI 95%. The blood was collected and then measured for cholesterol, triglyceride, LDL, and HDL levels. The results of the test showed that the administration of the three doses of CN was proven to reduce total cholesterol levels in rats, accompanied by a decrease in triglyceride and LDL levels, as well as an increase in HDL in the treatment for four weeks (28 days) and eight weeks (56 days) when compared to the control group and the HFD group. The results combine efficacious nutraceuticals showing their effectiveness in reducing lipidemia levels. The effective dose was the lowest, 54 mg/kgBW.

Keywords: combinative nutraceutical, in vivo, high-fat diet, lipidemia levels, physiological

P19-19

Dance Movement Therapy (DMT) for juvenile delinquents during the COVID-19 Pandemic: Focusing on psychophysiological changes in depression

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The aim of this study is to analyze the psychophysiological differences before and after participation in dance movement therapy (DMT) between juvenile delinquents and adolescents in general school in the COVID-19 situation. A total of 42 female students, including a general youth control group and a juvenile delinquent group, participated in this study. The Beck Depression Inventory (BDI) scale, a psychological test, was performed before and after the DMT, and serotonin (5-HT) and Cortisol were analyzed as physiological indicators by blood tests. As a result, the juvenile delinquent group showed a significantly decreased score on the depression scale BDI after 12 weeks of dance movement therapy, and there were statistically significant differences in cortisol and 5-HT between the general juvenile control group and the juvenile delinquent group. Although it is difficult to generalize the results of this study, DMT was found to be effective in lowering the degree of depression caused by COVID-19 in juvenile delinquents according to the BDI results, and also significantly changed 5-HT, a cortisol.

Keywords: dance movement therapy (DMT), juvenile delinquents, depression amid COVID-19, cortisol

P19-20

Mediation of lateral hypothalamus orexin input to lateral habenula in the inhibitory effects of mechanical stimulation on psychomotor responses induced by cocaine

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Background: The lateral hypothalamus plays an important physiologic role in brain function and also plays an important role in substance abuse. The neuropeptides called orexin (or hypocretins) have been identified as being located exclusively in cellular bodies of lateral hypothalamus (LH). Our previous studies have demonstrated that mechanical stimulation (MS) of ulnar nerve produces strong inhibitory effects on cocaine addiction-like behaviors through activation of LH projecting to lateral habenula. Therefore, the present study hypothesized that ulnar MS would suppress the psychomotor responses induced by cocaine through orexinergic LH-to-LHb pathway.
Methods: Locomotor activity (LOCO) and 50-kHz ultrasonic vocalizations (USVs) were investigated to confirm the effect of MS stimulation in acute cocaine-induced rats. Whether the artificial orexin increase suppresses cocaine-induced psychomotor response was investigated through LOCO and 50-kHz USVS. MS used in vivo extracellular recordings to determine whether LHb neurons are activated via orexin.
Results: Ulnar MS attenuated cocaine enhancement of locomotor activity and 50-kHz USVs, which was prevented by antagonism of OX2R in LHb. Injection of orexin-A into LHb reduced the cocaine-induced psychomotor responses. MS of ulnar nerve excited LH neuron. In addition, excitation of LHb neurons by MS was blocked by systemic administration of OX2R antagonist.
Conclusion: These findings suggest that MS applied to the ulnar nerve recruits an orexinergic LH-to-LHb pathway to suppress psychomotor responses induced by cocaine.

Keywords: orexin, orexin antagonist, mechanical stimulation, cocaine, lateral hypothalamus

P19-21

Effects of music therapy as an alternative treatment on depression in children and adolescents with ADHD by activating serotonin and improving stress coping ability

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The objective of this study was to determine the effect of music therapy as an alternative treatment on depression in children and adolescents with attention-deficit hyperactivity disorder (ADHD) by activating serotonin (5-HT) and improving stress coping ability. The ADHD control group received standard care, while the ADHD music therapy group received music therapy and standard care. The ADHD music therapy group received both active music therapy (improvisation) and receptive music therapy (music listening) for 50 minutes, twice a week, for three months: a total of 24 times. The ADHD music therapy group's 5-HT secretion increased whereas cortisol expression BP and HR decreased. However, the ADHD Con G's 5-HT secretion did not increase, whereas cortisol expression, BP, and HR did not decrease. Therefore, this study would like to propose a new alternative to medicine for preventing and treating depression through various uses of music therapy.

Keywords: children and adolescents with ADHD, music therapy, serotonin (5-HT), cortisol, depression

P19-22

The effect of cytotoxic compounds from nutmeg seed (myristica fragrans) induces apoptosis in melanoma cancer cell's (B16-F10)

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Melanoma is a type of skin cancer that occurs in the skin's pigment-producing cells, called melanocytes. This cancer has a high mortality rate, especially if it is not detected early. Indonesia is a country that rich in medicinal plants, one of which comes from the nutmeg plant (*Myristica fragrans*) which is known to contain secondary metabolites that have the potential to have cytotoxic activity. This study aims to obtain the chemical structure of cytotoxic compounds from seeds *M. fragrans* against melanoma cancer cells (B16-F10) and their activity against melanoma cancer cells (B16-F10), to analyze the cytotoxic compounds of seeds M. fragrans in inducing apoptosis signaling through the expression of caspase-9 and caspase-3 proteins in melanoma cancer cells (B16-F10) using in cell western. This research method includes the extraction of seeds *M.fragrans* using maceration techniques with ethanol and partitioned with n-hexane, ethyl acetate, and n-butanol 3respectively. Extracts and fractions were tested for cytotoxic activity in vitro against melanoma cells (B16-F10). The active fraction is then separated and purified by various chromatography techniques until three pure compounds were isolated. These compounds were characterized using spectroscopic methods (NMR, MS, UV, dan IR) and tested for cytotoxic activity in vitro against melanoma cells (B16-F10). Apoptosis signaling pathways through cytochrome c, caspase-9 and caspase-3 were tested using the in cell western. These three new novel compounds were identified as phenolic compound, [2,3-dihydro-2-(3-hydroxy-5-metoxyphenyl)-7metoxy-3-methyl-5-trans-prophenyl benzofuran] (1), Maceneolignan B (2), and Myristicin (3). Cytotoxic activity of the compounds (1-3) have a IC_{50} 61,93 ; 138,42; and 555,10 µM. The compound (1) induces apoptosis in melanoma cells (B16-F10) through increased proteins expression of cytochrome c, caspase-9 and caspase-3. Compound (1) from seeds *Myristica. fragrans* has the potential to be additional treatment for melanoma cancer.

Keywords: myristica fragrans, apoptosis, melanoma, [2,3-dihydro-2-(3-hydroxy-5-metoxyphenyl)-7metoxy-3-methyl-5-trans-prophenyl benzofuran, cytotoxic

P19-23

Exploring the role of propolis on lipid profile of wistar rats with induced dyslipidemia

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Obesity has now become a global epidemic with a high annual death rate. In many cases, it is accompanied by dyslipidemia which may further result in complications. To alleviate dyslipidemia, various alternative therapies have come to light, with propolis being one of them. However, the research of propolis effects on dyslipidemia is limited. Therefore, in this study, we aimed to analyze the effect of propolis supplementation in modulating lipid metabolism in HFD-model rats to explore its potential as an alternative treatment for HFD-induced obesity in the near future. To measure lipid profile, blood was collected from the tail of male Wistar rats aged 24 weeks which have been separated into 4 groups and undergone 12 weeks of obesity induction continued by propolis supplementation 300 mg/kg for 4 weeks, then analyzed for lipid profile after serum is separated. Using calorimetric assay, samples are mixed with Diasys reagent and run through Tecan Infinite M200 at a wavelength between 546 and 500 nm. The results showed that supplementation of propolis at a dose of 300 mg/kg for 4 weeks is effective in modulating lipid profile of HFD-induced dyslipidemia. This was portrayed by a significant decrease of TC level ($p \leq 0.001$) and TG level ($p \leq 0.01$), accompanied by a rise in HDL in HFDP group ($p \leq 0.001$). To summarize, propolis may serve as a highly potential alternative treatment for obesity and metabolic syndrome through pathways involved in dyslipidemia.

Keywords: propolis, dyslipidemia, high-fat diet, metabolism, lipid profile

Poster

P20-1

Safety assessment of fragmented polystyrene microplastics in ICR mice with single- and two week repeated administration

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Recently, microplastics are the main pollutant against environment and living organisms including human. To prevent and do preemptively before something rise up, the safety assessment of the microplastics should be conducted. In this study, we manufactured the polystyrene (PS) microplastics and toxicity studies using the microplastics were performed. We confirmed the manufactured particles were suitable with the definition of the microplastics through physical and chemical analysis and established the approximately lethal dose of the PS microplastics, 2,000 mg/kg or over.

Keywords: microplastics, polystyrene, toxicity, safety, approximately lethal dose

P20-2

Daidzein inhibits human platelet activation by downregulating thromboxane A2 production and granule release, regardless of COX-1 activity

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Platelets play crucial roles in cardiovascular diseases (CVDs) by regulating hemostasis and blood coagulation at sites of blood vessel damage. Accumulating evidence indicates daidzein inhibits platelet activation, but the mechanism involved has not been elucidated. Thus, in this study, we investigated the mechanism responsible for the inhibition of collagen-induced platelet aggregation by daidzein. We found that in collagen-induced platelets, daidzein suppressed the production of thromboxane A_2 (TXA_2), a molecule involved in platelet activation and aggregation, by inhibiting the cytosolic phospholipase A2 ($cPLA_2$) signaling pathway. However, daidzein did not affect cyclooxygenase-1 (COX-1). Furthermore, daidzein attenuated the PI3K/PDK1/Akt/GSK3αβ and MAPK (p38, ERK) signaling pathways, increased the phosphorylation of inositol trisphosphate receptor1 (IP_3R1) and vasodilator-stimulated phosphoprotein (VASP), and increased the level of cyclic adenosine monophosphate (cAMP). These results suggest that daidzein inhibits granule release (ATP, serotonin, P-selectin), integrin $\alpha IIb\beta_3$ activation, and clot retraction. Taken together, our study demonstrates that daidzein inhibits collagen-induced platelet aggregation and suggests that daidzein has therapeutic potential for the treatment of platelet aggregation-related diseases such as atherosclerosis and thrombosis.

Keywords: daidzein, cardiovascular disease, isoflavone, platelet activation, thromboxane A_2

P20-3

Transcutaneous auricular vagus nerve stimulation enhances cerebrospinal fluid circulation and restores cognitive function in the rodent model of vascular cognitive impairment

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Vascular cognitive impairment (VCI) is a common sequela of cerebrovascular disorders. Although transcutaneous auricular vagus nerve stimulation (taVNS) has been considered a complementary treatment for various cognitive disorders, preclinical data on the effect of taVNS on VCI and its mechanism remain ambiguous. To measure cerebrospinal fluid (CSF) circulation during taVNS, we used in vivo two-photon microscopy with CSF and vasculature tracers. VCI was induced by transient bilateral common carotid artery occlusion (tBCCAO) surgery in mice. The animals underwent anesthesia, off-site stimulation, or taVNS for 20 min. Cognitive tests, including the novel object recognition and the Y-maze tests, were performed 24 h after the last treatment. The long-term treatment group received 6 days of treatment and was tested on day 7; the short-term treatment group received 2 days of treatment and was tested 3 days after tBCCAO surgery. CSF circulation increased remarkably in the taVNS group, but not in the anesthesia-control or off-site-stimulation-control groups. The cognitive impairment induced by tBCCAO was significantly restored after both long- and short-term taVNS. In terms of effects, both long- and short-term stimulations showed similar recovery effects. Our findings provide evidence that taVNS can facilitate CSF circulation and that repetitive taVNS can ameliorate VCI symptoms.

Keywords: vascular cognitive impairment, vagus nerve stimulation, cerebrospinal fluid

P20-4

Cullin-RING E3 ubiquitin ligase 4 regulates neuritogenesis during neuronal development

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Neuritogenesis plays critical role in neuronal migration and connection during brain development. Thus, many neurodevelopmental disorders including intellectual disability is caused by deregulation of neuritogenesis. Cullin-RING E3 ubiquitin-ligase complexes have been proposed as a regulatory component for neurodevelopmental processes including neurite outgrowth via ubiquitin-proteasome system. In this study, we investigate a novel function of Cullin-RING E3 ubiquitin-ligase 4 (CRL4) for neurite morphogenesis during neurodevelopment. Cul4a and Cul4b, core scaffold molecules of CRL4, are highly expressed and neddylated in developing neuron. And they are down-regulated by neuronal activity via N-methyl D-aspartate receptor signaling. Notably, CRL4 interacts with cytoskeleton-regulating proteins, which are involved in neurite morphogenesis, such as Doublecortin. In addition, genetic perturbation of CRL4 induces changes on neurite extension and branching of developing neurons. Furthermore, CRL4 negatively regulates the stability of Doublecortin protein via ubiquitination-mediated degradation. Conclusively, we suggest that CRL4 works as a modulator for proper regulation of neurito-

genesis during neurodevelopment.

Keywords: Cullin-RING E3 ubiquitin ligase, neuritogenesis, neurodevelopment

P20-5

PCSK9 involves in the high-fat diet-induced abnormal testicular function of male mice

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Long-term consumption of a high-fat diet (HFD) is an important factor that leads to impaired spermatogenesis exhibiting poor sperm quantity and quality. However, the mechanism of which is yet to be elucidated. Disrupted cholesterol homeostasis is one of many crucial pathological factors which contributed to impaired spermatogenesis. As a negative regulator of cholesterol metabolism, preprotein invertase subtilin 9 (PCSK9) mediates LDLR degradation to the lysosome, thereby reducing the expression of LDLR on the cell membrane and increasing serum LDL-C level, resulting in lipid metabolism disorders. Here, we aim to study whether PCSK9 is a pathological factor of impaired spermatogenesis induced by HFD and the underlying mechanism of which, to meet the purpose of our study, we utilized wild-type of C57/B6 male mice and *PCSK9* knockout mice with same background as experimental subjects and alirocumab, a PCSK9 inhibitor, was used for treatment. Results indicated that HFD induced higher PCSK9 expression in serum, liver and testes, and serum PCSK9 is negatively correlated with spermatogenesis, while both PCSK9 inhibitor treatment and *PCSK9* knockout methodologies ameliorated impaired lipid metabolism and spermatogenesis of mice fed with HFD, which could be due to the over-expression of PCSK9 induced by HFD led to dyslipidemia, resulting in testicular lipotoxicity, thus activating the Bcl-2-Bax-Caspase3 apoptosis signaling pathway in testes, particularly in Leydig cells. Our study demonstrates that PCSK9 is an important pathological factor in the dysfunction of spermatogenesis in mice induced by HFD, which provides innovative ideas for the diagnosis and treatment of male infertility.

Keywords: PCSK9, LDLR, spermatogenesis, lipid metabolism

P20-6

CircHIPK3 targets DRP1 to mediate hydrogen peroxide-induced necroptosis of vascular smooth muscle cells and atherosclerotic vulnerable plaque formation

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Aims: Necroptosis induced by Hydrogen peroxide (H_2O_2) may play a vital role in atherosclerotic vulnerable plaque rupture, leading acute blood syndrome. However, the specific regulatory molecules of this development remain unclear. We aims to elucidate a mechanism from the perspective of circular RNA.
Results: We investigated that circHIPK3 was highly expressed in vulnerable plaques, and the increase in expression level promoted H_2O_2 induced necroptosis of VSMCs. CircHIPK3 targeted the protein DRP1, leading to an elevation in mitochondrial division rate, resulting in increased reactive oxygen species and impaired mitochondrial function, ultimately leading to necroptosis of VSMCs and vulnerable plaque formation.
Innovation: The finding of this study reveals and elucidates the specific molecular mechanisms in the formation of atherosclerotic vulnerable plaques and explores new molecular diagnostic targets for atherosclerotic plaque vulnerability on a clinical and cellular level.
Conclusion: CircHIPK3 interact with DRP1 involve in H2O2 induced Mitochondrial damage and necroptosis of VSMCs, and Silencing circHIPK3 in vivo can reduce atherosclerotic vulnerable plaque formation.

Keywords: necroptosis, atherosclerotic vulnerable plaque, circular RNA, mitochondrial division

P20-7

Mutational profiles of the BRCA1/2 transcripts in the Taiwanese women population

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Breast cancer is the most common cancer among women worldwide. Among all risk factors, pathogenic variants of breast cancer susceptibility gene 1 (BRCA1) and breast cancer susceptibility gene 2 (BRCA2) have been associated with the predisposition to breast and ovarian cancers. Current BRCA1/2 genetic tests commonly focus on the coding regions, and 50% of hereditary breast cancer cases failed to identify causal mutation after having BRCA1/2 genetic tests. Recent studies showed that dysregulation of alternative splicing (AS) is an important cause of tumorigenesis and microenvironment formation. Nevertheless, the role of BRCA1/2 AS transcripts in breast cancer initiation and development remains unclear. The diversity of pathogenic causes indicates an urgent need to develop a more comprehensive workflow to screen BRCA1/2 gene mutations for clinical practice. This study aims to develop an RNA-based, comprehensive workflow to screen BRCA1/2 gene mutations for clinical practice to meet clinical needs. Human primary ductal carcinoma lymphoblast cell line HCC1937 with a known insertion at c.5266 was used to establish the protocol. Primers were designed for amplicons covering entire BRCA1/2 exons. After next-generation sequencing (NGS), data was analyzed by an in-house developed pipeline to annotate sequencing results. We applied the workflow to 114 blood samples collected from a local Women and Children's Hospital between March 2023 and May 2023. In addition, pathogenic BRCA1/2 variants from the ClinVar database and BRCA1/2 AS events with indicated clinical significance from previous studies were collected and used to classify variant pathogenicity. We identified 48 SNPs and classified them into frameshift (N=3), in-frame indel (N=3), missense (N=29), and synonymous (N=13). Two SNPs are pathogenic variants, while the rest are variants with unknown significance (N=8) or benign/likely benign (N=38). Furthermore, 5 AS events, two on BRCA1 and 3 on BRCA2, were found. Whereas three commonly occurring AS events, BRCA1 Δexon4, BRCA1 Δexon8_9, and BRCA2 Δexon12 were identified in 9 (7.9%), 85 (74.6%), and 10 (8.8%) individuals, respectively, one pathogenic AS event has been reported to be related to disease (BRCA2 Δexon3, c.68_316del) and one AS event that was not reported before (BRCA2 Δexon9_10, c.682_1909del) with a predicted in-frame deletion. Collectively, this is the first study to report BRCA1/2 RNA mutations in Taiwanese women. We expect the established comprehensive and clinically feasible workflow will help to profile BRCA1/2 transcript mutations, especially the AS variants, in the Taiwanese population. Nevertheless, the clinical significance of

common AS variants merits further investigation.

Keywords: BRCA1/2, alternative splicing variant, genetic predisposition, genetic test, mutational profiles

P20-8

IL-10 and TNF α as paracrine effect of encapsulated mesenchymal stem cell coating by platelet lysate

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Mesenchymal stem cells (MSCs) have been used as a cellular therapy for infectious and degenerative diseases in the last decades because of their paracrine effect, immunomodulatory capability, high ability differentiation, and high plasticity. The paracrine effect of MSCs contains many growth factors and pro-inflammatory cytokines so that it can modulate the immune system. TNF-α and IL-10 regulate inflammatory reactions. TNF-α stimulates the release of many inflammatory cytokines, and IL-10 reduces pro-inflammatory signals. Nevertheless, there are many obstacles to maintaining paracrine effects in cellular therapy due to a shortage of cellular retention. MSC encapsulation offers a favourable environment for the survival rate of MSCs due to the increasing resistance to host immune cells. Platelet lysate contains many growth factors that can enhance MSCs' paracrine effect. In this study, MSCs were encapsulated with alginate, crosslinked by CaCl2, and subsequently coated with platelet lysate as non-coating platelet lysate was the control. Encapsulated MSCs were cultured for 21 days and analyzed for IL-10 and TNF-α levels. Our study showed that TNF-α levels in encapsulated MSCs coated by platelet lysate increased on day seven and maintained until day 21. In contrast, TNF-α levels' non-coating platelet lysate decreased on day seven and was almost undetected on day 21. Meanwhile, IL-10 was maintained until day 14 in non-coating encapsulation and kept inside the capsule in coating until day 14. This study showed that encapsulated MSCs coated with platelet lysate affected MSCs' paracrine effect in TNF-α levels and retained IL-10 inside the capsule.

Keywords: IL-10, TNF-α, MSC, encapsulated, platelet lysate

P20-9

A novel multimodal flexible polyimide neural probe for neural signal recording in mouse

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Flexible neural probes with low impedance electrodes are an attractive emerging technology for brain recordings, given that they can effectively measure signals with minimized risks of brain damage. Neural probes with multiple modalities offer a potent approach to investigating neural circuit functions and gaining insights into brain diseases. Here, we fabricated a novel multimodal flexible polyimide neural probe and evaluated its ability to measure the activity of cells in the mouse brain region.

A flexible polyimide neural probe coated with electrodeposited platinum was integrated with electrophysiology recoding system. The probe wasacutely inserted into the brain of anesthetized and awake mouse. Electrical signals of neurons in the target region, the thalamus, were measured with 32 electrodes at impedance values of 50 KΩ, 250 KΩ, 500 KΩ, and 1000 KΩ at 1 kHz. We investigated the optimal impedance for measuring the electrical signals of neurons and distinguishing cell clusters. Subsequently, we assessed its ability to record under chronic condition and during in vivo optogenetic stimulation.

The probes showed significant flexibility, which resulted in no instances of mechanical or electrical failure during repeated brain insertions. In the anesthetized mice, the 250 KΩ impedance probe effectively measured action potentials and distinguished cell clusters. The 50 KΩ impedance probe could measure action potentials, but it did not effectively classify cell clusters. Probes with 500 KΩ and 1000 KΩ impedances could classify cell clusters, but they were not efficient in measuring action potentials. In the awake mice, the probe allowed its longevity of high-quality recording for 4 weeks. Furthermore, the probe was able to record neural activity of opsin-expressing cells during in vivo optical stimulation using pulses of light at a specific wavelength.

In conclusion, our flexible multimodal polyimide neural probe successfully measured neuronal signals and classified cell clusters. Our findings suggest the potential presence of impedance values optimized for measuring specific brain regions. Furthermore, we demonstrated its capability for stable, long-term electrophysiological recording and optogenetic analysis. It indicates that the probes can be utilized as versatile neural probes for various applications.

Keywords: flexible neural probe, cellular activity, electrical recording, mouse brain

P20-10

CR6 interacting factor 1 deficiency increased homocysteine production by suppressing dihydrofolate reductase expression in vascular endothelial cells

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Elevated plasma homocysteine levels can induce vascular endothelial dysfunction; however, the mechanisms regulating homocysteine metabolism in impaired endothelial cells are currently unclear. In this study, we deleted the essential mitoribosomal gene CR6 interacting factor 1 (CRIF1) in human umbilical vein endothelial cells (HUVECs) and mice to induce endothelial cell dysfunction; then, we monitored homocysteine accumulation. We found that CRIF1 downregulation caused significant increases in intracellular and plasma concentrations of homocysteine, which were associated with decreased levels of folate cycle intermediates such as 5-methyltetrahydrofolate (MTHF) and tetrahydrofolate (THF). Moreover, dihydrofolate reductase (DHFR), a key enzyme in folate-mediated metabolism, exhibited impaired activity and decreased protein expression in CRIF1 knockdown endothelial cells. Supplementation with folic acid did not restore DHFR expression levels or MTHF and homocysteine concentrations in endothelial cells with a CRIF1 deletion or DHFR knockdown. However, the overexpression

of DHFR in CRIF1 knockdown endothelial cells resulted in decreased accumulation of homocysteine. Taken together, our findings suggest that CRIF1-deleted endothelial cells accumulated more homocysteine, compared with control cells; this was primarily mediated by the disruption of DHFR expression.

Keywords: CR6 interacting factor 1, dihydrofolate reductase, folic acid, homocysteine

P20-11

Hypothalamic SF1-expressing neurons encode a conspecific-tuned, sex- specific behavioral state that modulates social investigation

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The ventromedial hypothalamic nucleus (VMH) is vital for various innate behaviors. Among multiple neural subsets within the VMH, the estrogen-receptor-1-expressing neurons in the ventrolateral division (VMHvl) are well-known for their involvements in consummatory social behaviors (aggression, mating, self-defence). Another distinct dorsomedial VMH (VMHdm) residing VMH neural subset could be discerned by the expression of a transcription factor, steroidogenic factor-1 (SF1). Studies showed that VMHdm^{SF1} neurons encode a predator- orientated defensive state. Nevertheless, neuroanatomical features suggested that the VMHdm^{SF1} neurons receive inputs from other social-related VMH neural subpopulations, and the VMHdm^{SF1} neurons may receive and process social-related sensory cues. To address the functional involvement of VMHdm^{SF1} neurons in sculpting social behaviors, we performed cell-type-specific in vivo calcium imaging in freely roaming Sf1-Cre transgenic mice. We revealed that the VMHdm^{SF1} neurons were robustly activated by social- but not predator- associated stimuli, with a male-biased conspecific sex representation. In addition, conspecifics with different sexes selectively recruited distinct VMHdm^{SF1} neural subsets. Through selective ablation of particular olfactory transmitting pathways, we found that male-biased populational responses of the VMHdm^{SF1} neurons depend on pheromonal signals, which are majorly transmitted from VNO to hypothalamic circuit through the bed nucleus of stria terminalis (BNST). By optogenetically silencing the BNST-VMHdm pathway, we could diminish the male-preference among the VMHdm (putative SF1-expressing) neural population. Moreover, VMHdm^{SF1} neuronal activities were highly correlated with social investigative behaviors. Altogether, we proposed that apart from the defensive behavioral state, a large portion of VMHdm^{SF1} neurons are capable of encoding conspecific social cues. The conspecific sex representation of VMHdm^{SF1} neurons may prompt animals' invesitgation upon encountering other mice, which facilitates proper behavioral decision making via accessing more information.

Keywords: hypothalamus, VMH-SF1 neurons, bed nucleus of stria terminalis, social behaviors, calcium imaging

P20-12

Differential expression of ORAI channels and STIM proteins in renal cell carcinoma subtypes: Implications for metastasis and therapeutic targeting

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ORAI1 and STIM1 are essential molecular components of store-operated calcium entry (SOCE), playing critical roles for cell migration and proliferation within clear cell renal cell carcinoma (ccRCC). While the significance of SOCE in tumour growth and metastasis is well-documented, its exploration in other renal cancer subtypes remains limited. In this study, we delve into SOCE-associated gene expression and the pathophysiological significance of these genes across various subtypes of RCC.

Through an analysis of the TCGA Pan-Kidney Cancer Atlas, which encompasses clear cell (n= 534) and papillary (n = 291) renal cancer types, we observe highlighted expression of ORAI channels (ORAI1-3) and STIM1, juxtaposed against normal levels, in both cancer types. However, we note a conspicuous decrease in STIM2 expression in papillary RCC (pRCC). Further inspection of FPKM (fragments per kilobase million) data from the TCGA database reveals a significant upregulation of ORAI3 in pRCC relative to ccRCC across all cancer stages. Notably, a negative correlation between ORAI3 and STIM2 is prominent in the majority of pRCC patients. In parallel, diverse expression patterns of ORAI1 and ORAI3 are identified in RCC cell lines, with ORAI3 dominating in Caki-2 (pRCC primary cell line) and ORAI1 in Caki-1 (ccRCC metastatic cell line). Enhanced SOCE via 2-APB underscores ORAI3 as the primary calcium channel in Caki-2, whereas reduced SOCE in Caki-1 suggests ORAI1's prevalence. Additionally, the functional knockdown of ORAI1 and 3 impedes cell migration in both cell lines, accentuating their indispensable roles.

Collectively, our findings underscore the contributory roles of over-expressed ORAI3 and ORAI1 in RCC metastasis. Specifically, the augmented ORAI3 expression accompanied by reduced STIM2 levels in pRCC offers novel insights into molecular subtypes, potentially guiding diagnostic and therapeutic avenues. This investigation sheds light on the potential targeting of ORAI channels and STIMs to tackle RCC progression and metastasis.

Keywords: Orai1, Orai3, STIM1, STIM2, renal cell carcinoma

P20-13

Secretion of APE1/Ref-1 and its role in vascular inflammation

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Apurinic/apyrimidinic endonuclease 1/redox factor-1 (APE1/Ref-1) is an essential multifunctional protein involved in DNA base repair and reducing activity. Several studies have demonstrated the usefulness of APE1/Ref-1 as a serological biomarker. However, the role of APE1/Ref-1 in vascular inflammation is unclear. The aim of this study was to investigate the role of secreted APE1/Ref-1 in vascular inflammation using mouse

model of atherosclerosis and sepsis. The endothelial/macrophage activation as vascular inflammation, and atherosclerotic plaque was evaluated in ApoE-/- mice fed Western type diet. We examined the serologic APE1/Ref-1 was correlated with vascular inflammation. Next, we investigated the role of APE1/Ref-1 on lipopolysaccharide (LPS)-induced vascular inflammation using designed secretory APE1/Ref-1, AdPPT-LS-Ref-1. APE1/Ref-1 expression was markedly increased in the aortic tissue of ApoE-/- mice fed Western type diet, and this expression was co-localized with CD31 and Galectin-3, suggesting the endothelial/macrophage expression of APE1/Ref-1. Surprisingly, the levels of plasma APE1/Ref-1 in ApoE-/- mice fed Western type diet showed a significant increase compared to those in normal diet group. Treatment with LPS markedly increased VCAM-1 expression, cathepsin or myeloperoxidase activity, which were significantly suppressed by treatment with secretory APE1/Ref-1. Taken together, APE1/Ref-1 expression is upregulated in atherosclerotic plaque, and plasma APE1/Ref-1 levels may predict atherosclerotic inflammation. Furthermore, our findings indicate that secretory APE1/Ref-1 may be useful as a therapeutic biomolecule against vascular inflammation.

Keywords: APE1/Ref-1, vascular inflammation, atherosclerotic mice, septic mice

Poster

P20-14

Electroacupuncture alleviates ulcerative colitis by targeting CXCL1: evidence from the transcriptome and validation

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Background: We aimed to use transcriptomics, bioinformatics analysis, and core gene validation to identify the core gene and potential mechanisms for electroacupuncture (EA) treatment of ulcerative colitis (UC).
Materials and methods: EA was performed in mice after induction of UC via dextran sodium sulfate. Body weight, disease activity index (DAI), colon length, and hematoxylin-eosin of the colon tissue were used to evaluate the effects of EA. Mice transcriptome samples were analyzed to identify the core genes, and further verified with human transcriptome database; the ImmuCellAI database was used to analyze the relationship between the core gene and immune infiltrating cells (IICs); and immunofluorescence was used to verify the results.
Results: EA could reduce DAI and histological colitis scores, increase bodyweight and colon length, and improve the expression of local and systemic proinflammatory factors in the serum and colon of UC mice. Eighteen codifferentially expressed genes were identified by joint bioinformatics analyses of mouse and human transcriptional data; Cxcl1 was the core gene. EA affected IICs by inhibiting Cxcl1 expression and regulated the polarization of macrophages by affecting the Th1 cytokine IFN-g, inhibiting the expression of CXCL1.
Conclusions: CXCL1 is the target of EA, which is associated with the underlying immune mechanism related to Th1 cytokine IFN-g.

Keywords: ulcerative colitis, electroacupuncture, transcriptome, CXC motif chemokine ligand 1, immune infiltrating cells

P20-15

The zinc transporter ZIP7 plays a critical role in ferroptosis induced by ischemia/reperfusion in mouse hearts

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Background: Although ferroptosis has been proposed to play a role in myocardial ischemia/reperfusion (I/R) injury, the exact molecular mechanism by which ferroptosis is induced by I/R remains unclear. To determine if ZIP7 plays a role in I/R-induced ferroptosis and to investigate the underlying molecular mechanism. We tested the hypothesis that upregulation of the zinc transporter ZIP7 at reperfusion triggers ferroptosis by downregulating the mitochondrial GPX4 (mtGPX4) via the guanine-rich sequence-binding factor 1 (GRSF1) and increasing mitochondrial iron accumulation through inhibition of the mitochondrial iron importer degradation.
Methods: Mouse hearts were subjected to I/R *in vivo*. The cardiac-specific*Slc39a7* conditional knockout mice (ZIP7 cKO) were generated by adopting the CRISPR/Cas9 system. Adult mouse cardiomyocytes were isolated enzymatically. Ferroptosis was evaluated by detecting PTGS2, MDA, and BODIPY C11. Mitochondrial Fe^{2+} was determined with Mito-FerroGreen fluorescent probe.
Results: I/R upregulated ZIP7 expression and induced ferroptosis as indicated by the increases in PTGS2 mRNA expression (290.90 ± 52.78 % of control), MDA (152.10 ± 15.07 % of control) and BODIPY C11 (130.68 ± 5.52 % of control) levels, which was prevented by ZIP7 cKO. ZIP7 cKO also mitigated doxorubicin-induced ferroptosis. ZIP7 cKO upregulated mtGPX4 expression (123.40±3.26 % of I/R) and increased mitochondrial GRSF1 expression (150.90±8.51 % of I/R) at reperfusion. In addition, knockout of GRSF1 inhibited GPX4 expression (54.73±5.44 % of control). Moreover, ZIP7 cKO prevented the accumulation of Fe^{2+} within mitochondria (44.15±2.46 % of I/R) and promoted the degradation of SLC25A37 (62.57±8.03% of I/R) and SLC25A28 (69.82±3.10 % of I/R) at reperfusion.
Conclusions: Upregulation of ZIP7 contributes to the induction of ferroptosis at reperfusion presumably by reducing mtGPX4 expression via GRSF1and increasing Fe^{2+}accumulation within mitochondria through inhibition of SLC25A37 and SLC25A28 degradation.

Keywords: myocardial ischemia/reperfusion, ferroptosis, ZIP7, GPX4, GRSF1

P20-16

Electroacupuncture for chronic neuropathic pain on astroglial glutamate-glutamine

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Background: Based on our previous study, we observed that astrocytes are involved in the process of cumulative analgesia, but it's underly mechanism is unclear. Glutamate is extensively recycled between neurons and astrocytes, a process known as the glutamate-glutamine cycle. Therefore, in this study, we investigated the effect of EA on astroglial glutamate-glutamine in the spinal cord dorsal horns in neuropathic pain rats.
Methods: 48 male SD rats were randomly divided into normal control

group, chronic constriction injury(CCI) group, and electroacupuncture (EA) group. EA was applied to bilateral Zusanli(ST36)-Yanglingquan(GB34). The neuropathic pain model was established by sciatic nerve-ligation. The mechanical and thermal pain thresholds of bilateral hind paws were measured. The co-expression of spinal glutamate/aspartate transporter (GLAST) with microglia and astrocytes was detected by immunofluorescence double-labeling. Western blot and PCR detected the protein and mRNA expression of spinal GLAST, glutamate transporter-1 (GLT-1), glutamine synthetase(GS), p-ERK. The contents of glutamate (Glu) and gamma-aminobutyric acid (GABA) in the spinal cord were measured by the high-performance liquid phase (HPLC) method. To further verify the effects of GLAST and GLT-1 in EA analgesia, another 30 male SD rats were randomly divided into the following three groups: intrathecal saline injection + EA group, intrathecal GLAST antagonist injection + EA group, and intrathecal GLT-1 antagonist injection + EA group.

Results: After CCI, both mechanical and thermal pain scores of the hind paws were significantly increased($P<0.05$), indicating a significant decrease in mechanical and thermal pain thresholds . CCI also significantly decreased the proteins and mRNA expression of GLAST and GLT-1 ($P<0.05$), slightly decreased the proteins and mRNA expression of GS ($p>0.05$), but significantly increased the content of Glu. After EA treatment, the mechanical and thermal pain thresholds of the bilateral hind paws were significantly increased ($P<0.05$), and the proteins and mRNA expression of GLAST and GLT-1 was increased considerably, and GS was incrseased slightly, the content of Glu was decreased. After intrathecal injection of glutamate transporter antagonist, both mechanical and thermal radiation pain thresholds of rats were significantly decreased ($P<0.05$), suggesting that the analgesic effect of EA was reversed under the glutamate transporter antagonist .

Conclusion: Repeated EA can inhibit CCI-induced chronic neuropathic pain in rats, which may be related to reversing the decrease in the expression of GLAST and GLT-1 in astrocytes, accelerating the clearance of Glu, thereby reducing the concentration of Glu. The possible target of cumulative EA analgesia in glutamate-glutamine is the regulation of glutamate transporters on astrocytes.

Keywords: chronic neuropathic pain, GLAST, GLT-1, electroacupuncture, glutamate transporters

P20-17

Spatial transcriptomics shows moxibustion promotes hippocampus astrocyte and neuron interaction

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Aims: Alzheimer's disease (AD) is a common and irreversible neurodegenerative disease accompanied by extensive synaptic loss. Previous studies found that moxibustion had good therapeutic effects on AD. We here investigated whether moxibustion could alleviate the cognitive impairment of AD by promoting the "astrocyte-neuron" interaction and enhancing synaptic plasticity.

Materials and methods: Moxibustion treatment was administrated to Baihui (GV20) and Yongquan (KI1) in APP/PS1 mice. We first evaluated the behavior of APP/PS1 mice with Morris water maze test, and observed the synaptic structure before and after moxibustion intervention. Then, the transcriptome characteristics (TC) and "astrocyte-neuron" interaction were evaluated by spatial transcriptomics (ST). CD38 and its ligand Pecam1, one of the energy shuttle pathways between neurons and astrocytes, were also be detected.

Key findings: The results supported that moxibustion increased learning and memory ability and synaptic structure. ST showed that the TC were more similar between the moxibustion and control groups. Moxibustion enhanced the number of ligand - receptor pairs between astrocytes and neurons. And the score of interaction intensity and the proportion of interaction were also increased. Meanwhile, the energy of astrocytes and neurons was significantly altered. Additionally, moxibustion could significantly improve the function of CD38 and its ligand Pecam1 which were previously reported having the function of transporting mitochondria from astrocytes to neurons, and then providing energy for neurons.

Keywords: spatial transcriptomics, moxibustion, neuron, astrocyte, interaction

P20-18

Residues in the rib helix of TRPC4 regulate conformation and transmission of G protein activation signal to channel gating

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The canonical group of transient receptor potential (TRPC) proteins form Ca^{2+}-permeable cation channels that respond to G protein signaling. Among them, TRPC4 is activated by pertussis toxin-sensitive $G_{i/o}$ proteins. Previous studies have revealed the importance of $G\alpha_{i/o}$ subunits in TRPC4 gating and implicated the involvement of the highly conserved CaM- and IP_3R-binding (CIRB) domain at the cytoplasmic C-terminus of TRPC4 for the G protein regulation. Using mutational studies and homology modeling, we show here that the C-terminal half of TRPC4 CIRB domain adapts an amphipathic organization with positively charged residues facing the inter-protomer space and hydrophobic residues interacting with the N-terminal linker helices from the same protomer. While the hydrophobic residues, Tyr706, Met710 and Tyr717, contribute to maintaining structural stability, the basic residues, R711, K715, and R716, on the opposite side of the rib helix help transmit the G protein binding signal to channel gating. Whereas charge neutralization of R711, K715, and R716 strongly facilitated, the mutation to opposite charge of these residues suppressed $G_{i/o}$-mediated TRPC4 activation. From a recently revealed architecture in which $G\alpha_{i3}$ is bound to the ankyrin repeat edge of TRPC5 channel, we propose an extended mode for activation in which $G\alpha_{i/o}$ binding strongly influences the electrostatic environment near the CIRB domain.

Keywords: TRP, helix, domain, G protein

P20-19

Immune modulation through synergistic interplay of gamma irradiation and interleukin-33 sensitization in mice-bearing EMT6 tumor model

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The immune response within the tumor microenvironment (TME) can be modulated by radiotherapy, resulting in an anti-tumor effect. Various immunomodulatory proteins, including IL-33, present within

the TME have been found to play a role in pro and antitumorigenic activities mediated by immune cells, such as eosinophils. A recent study suggested that IL-33, influenced by eosinophils, can inhibit tumor growth in a colorectal model. Therefore, the aim of this study was to investigate how radiation and IL-33 interact to modulate immune cells within TME, and their effects on the immune population and tumorigenic activity. To achieve this, 36 mice-bearing tumor models from EMT-6 cells inoculation were sensitized with IL-33 alone or with a combination of 2 or 8 Gy gamma irradiation, and then divided into early (96 hrs PRT) and acute (192 hrs PRT) phase studies. The results showed that the mice tumor model sensitized to IL-33 exhibited systemic neutrophilia and lymphopenia instead of inducing eosinophils. When exposed to 8 Gy gamma irradiation, the sensitized tumor model displayed a comparable immunosuppressive effect to the non-sensitized tumor model, except for a significant but temporary reduction in systemic neutrophils. There was a significant increase in CD45+ cells and neutrophils within the TME during the acute phase compared to the early phase across the sensitized tumor models, regardless of whether irradiation treatment was administered or not. The same tumor model also demonstrated significantly slower tumor growth, which was hypothesized to result from a synergistic effect resulting from IL-33 sensitization and targeted irradiation of 8 Gy.

Keywords: irradiation, interleukin-33, tumor microenvironment, immune modulation, tumorigenic activity

Poster

P20-20

Lubiprostone promotes intestinal motility by activating the TRPC4 channel in the colonic myocyte

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Prokinetic agents enhance gastrointestinal (GI) contractility to improve the symptoms of patients with delayed intestinal motility, typically constipation. Lubiprostone is known to activate Prostaglandin E2 (PGE2) receptors and chloride channel type 2 (CLC-2) channels, thereby increasing the chloride ion content in the GI tract, leading to enhanced water retention. However, the mechanism underlying muscle contraction by which it acts on colonic muscles remains largely unknown. Here, we focus on the role of the distal colon, proposing that lubiprostone ameliorates constipation by targeting canonical transient receptor potential cation channel type 4 (TRPC4) involved in colonic contraction.

First, in murine colonic tension recording, the application of lubiprostone notably augmented the contraction of distal longitudinal muscles compared to the proximal ones. To investigate the contrasting responses between the proximal and distal colon, we measured the mRNA levels of TRPC, CLC-2, and PGE2. Notably, TRPC4 and TRPC6 showed heightened expression in the distal colon compared to the proximal. Lubiprostone only increased the whole-cell current of TRPC4. The cellular calcium concentration, elevated by lubiprostone, diminished in the presence of Pico145, a selective agonist of TRPC4, a trend also observed in primary cultured murine colonic myocytes. Consequently, the activation of the TRPC4 channel by lubiprostone enhances colonic contraction.

Therefore, we propose that lubiprostone can exert potential efficacy on the fluidity and contractility in gut on site-by-site. These findings reveal a novel mechanistic insight into colonic contraction via the TRPC4 channel activation by lubiprostone. This understanding not only suggests therapeutic strategies targeting the contraction mechanism but also reshapes our perspective on functional constipation, suggesting a broader spectrum of intervention possibilities beyond conventional treatments.

Keywords: lubiprostone, TRPC4, gastrointestinal contractility, smooth muscle, motility

P20-21

Low concentrations of tricyclic antidepressants stimulate TRPC4 channel activity by acting as an opioid receptor ligand

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Traditionally prescribed for mood disorders, tricyclic antidepressants (TCAs) have shown promising therapeutic effects on chronic neuralgia and irritable bowel syndrome. However, the mechanism by which these atypical effects manifest is unclear. Among the proposed mechanisms is the well-known pain-related inhibitory G-protein coupled receptor (GiPCR), namely, the opioid receptor (OR). Here, we confirmed that TCA indeed stimulates OR and regulates the gating of TRPC4, a downstream signaling of the G_i-pathway. In an ELISA to quantify the amount of intracellular cAMP, a downstream product of OR/G_i-pathway, treatment with amitriptyline (AMI) showed a decrease in [cAMP]i similar to that of the μOR agonist. Next, we explored the binding site of TCA by modeling the previously revealed ligand-bound structure of μOR. A conserved aspartate residue of ORs was predicted to participate in salt bridge interaction with the amine group of TCAs, and in aspartate-to-arginine mutation, AMI did not decrease the FRET-based binding efficiency between the ORs and $G\alpha_{i2}$. As an alternative way to monitor the downstream signaling of G_i-pathway, we evaluated the functional activity of TRPC4 channel, as it is well known to be activated by $G\alpha_i$. TCAs increased the TRPC4 current through ORs, and TCA-evoked TRPC4 activation was abolished by an inhibitor of $G\alpha_{i2}$ or its dominant-negative mutant. As expected, TCA-evoked activation of TRPC4 was not observed in the aspartate mutants of OR. Taken together, OR could be proclaimed as a promising target among numerous binding partners of TCA, and TCA-evoked TRPC4 activation may help to explain the nonopioid analgesic effect of TCA.

Keywords: bias-agonism, opioid receptor, structure modeling, tricyclic antidepressants, TRPC4

P20-22

17β-Estradiol induces APE1/Ref-1 secretion in vascular endothelial cells though calcium-dependent exosome pathway

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Apurinic/apyrimidinic endonuclease-1/redox factor-1 (APE1/Ref-1) is a multifunctional protein that can be secreted, and recently suggested as new biomarker for vascular inflammation. Therefore, the current study aimed to identify potential endogenous hormones that increase APE1/Ref-1 secretion in vascular endothelial cells under conditions

that do not induce cell death; it also aimed to reveal the underlying secretion mechanism.The endogenous hormones that significantly increased APE1/Ref-1 secretion was 17β-estradiol (E2), 5alpha-dihydrotestosterone, progesterone, insulin, and insulin-like growth factor. The most potent hormone inducing APE1/Ref-1 secretion was E2, which in cultured endothelial cells, E2 for 24h increased APE1/Ref-1 secretion level of 4.56 ± 1.16 ng/mL, compared to a basal secretion level of 0.09 ± 0.02 ng/mL. Among the estrogens, only E2 increased APE1/Ref-1 secretion, not estrone and estriol. Blood APE1/Ref-1 concentrations decreased in ovariectomized (OVX) mice but were significantly increased by the replacement of E2 (0.39 ± 0.09 ng/mL for OVX vs. 4.67 ± 0.53 ng/mL for OVX + E2). E2-induced APE1/Ref-1secretion was remarkably suppressed by the estrogen receptor blocker and intracellular Ca^{2+} chelator, suggesting E2-induced APE1/Ref-1 secretion was dependent on ER and intracellular calcium. E2-induced APE1/Ref-1 secretion was significantly inhibited by exosome inhibitor GW4869. Furthermore, APE1/Ref-1 level in CD63-positive exosome were increased by E2. Finally, fluorescence imaging data showed that APE1/Ref-1 co-localized with CD63-labled exosome in the cytoplasm of cells upon E2 treatment. E2 was the most potent hormone for APE1/Ref-1 secretion, which appeared to occur through exosomes that were dependent on ER and intracellular Ca^{2+}. Furthermore, hormonal effects should be considered when analyzing biomarkers for vascular inflammation.

Keywords: 17β-estradiol, APE1/Ref-1, endothelial cells, exosome, estrogen receptor

P20-23

Magnetothermal brain stimulation modulates synaptic plasticity of the primary somatosensory cortex in adult mice

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Magnetic brain stimulation based on nanomaterials has attracted considerable interest in recent years as it is minimally invasive and can precisely regulate the activity of neuronal circuits without significant attenuation of magnetic strength. That is, the nanomaterial-dependent magnetic stimulation (NDMS) usually stimulates the targeted brain regions via a magneto-mechanical or magnetothermal force generated by the magnetic nanomaterials to activate exogenously-encoded Piezo1 or TRPV1, respectively, which convert magnetic energy into neural signals. Previous studies have demonstrated that these magnetophysical stimulations can induce neural excitation and elicit motor behavior in awake mice. However, these studies focused primarily on the direct and acute effect of the magnetophysical stimulation of the targeted neural activity, which directly regulates specific motor behaviors. In addition, they showed the impact of magnetophysical stimulation only in the presence of the exogenously expressed ion channel protein, which could pose a significant regulatory obstacle to clinical human translation. Here, we examined whether long-term nano-based magnetothermal stimulation (MTS) can achieve long-term synaptic plasticity *in vivo* configuration without any exogenous genetic modulation by electrophysiological methods. Nanoparticles (Synomag-D, micromod) were injected into the primary somatosensory cortex of adult mice aged eight weeks using a mini-osmotic pump for three days. Seven days after the nano injection, the mice were subjected to magnetic stimulation for 35 minutes daily for three consecutive days. The MTS immediately increased the spike rate of the primary somatosensory cortex. Moreover, the long-term MTS for three days increased field potentials of layer 4 of the primary somatosensory cortex evoked by whisker stimulation, indicating that MTS may modify the synaptic plasticity of the thalamorecipient layer 4 of the barrel cortex in adult mice. This study first observed that MTS can induce long-term cortical synaptic plasticity without any exogenously expressed channel proteins in adulthood.

Keywords: magnetothermal stimulation, nanoparticle, barrel cortex, synaptic plasticity, brain stimulation

P20-24

Amplification effect of exercise on intermittent fasting impact to modulate body composition in obese females

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Lifestyle modification by regulating diet and exercise is the right strategy to suppress the increase in obesity, because it can trigger fat browning resulting in energy expenditure in the form of heat (thermogenesis). This study aims to compare the effects of intermittent fasting (IF) and the combination of IF with exercise on body composition in obese females. A total of 16 obese female are the subjects of this study and will be randomly divided into 2 groups. The subjects were randomly divided into 2 groups, namely IFG (intermittent fasting group, n = 8) and IFEXG (intermittent fasting and exercise group, n = 8). Intermittent fasting was done for 12 hours, and exercise was done 30 minutes/session before breakfast. Exercise (running on a treadmill) was performed with an intensity of 60-70% HRmax, frequency 5x/week for 2 weeks. Body composition measurements were carried out on two resistances (pre- and post-treatment) using the TANITA Body Composition Analyzer DC-360. The data analysis technique used the paired sample t-test with a significance level of 5%. The results showed that there were no significant differences in body composition parameters between pre- and post-treatment on IFG. Meanwhile, weight loss (-2.05 kg; p=0.001), BMI (-0.84 kg/m2 ; p=0.001), FAT (-2.05 %; p=0.001), FM (-1.53 kg; p=0.001), FFM (-1.65 kg; p=0.001), and an increase in MM (1.20 kg; p=0.001) was observed in the IFEXG group between pre- and post-treatment. This study proves that the combination of IF and aerobic exercise for 2 weeks was effective in improving body composition in obese females.

Keywords: body composition, exercise, healthy lifestyle, intermittent fasting, obesity

P20-25

The APE1/Ref-1 induces anti-adipogenic function by inhibiting adipocyte differentiation in 3T3-L1

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The growth of adipose tissue involves an increase in adipocyte size

and differentiation of adipocytes from pre-adipocyte precursor cells. Under conditions of excess body fat and obesity, the body accumulates excessive amounts of triglycerides in adipocytes, which increases the triglyceride content of the liver, muscle, adipose tissue, and plasma, leading to pathological dysfunctions, such as metabolic syndrome, coronary heart disease, hypertension, type 2 diabetes, cancer, and osteoarthritis.
Apurinic/apyrimidinic endonuclease 1/redox factor-1 (APE1/Ref-1) is a multifunctional protein involved in DNA repair and redox regulation. The redox activity of APE1/Ref-1 is involved in inflammatory responses and regulation of DNA binding of transcription factors related to cell survival pathways. However, the effect of APE1/Ref-1 on adipogenic transcription factor regulation remains unknown. In this study, we investigated the effect of APE1/Ref-1 on the regulation of adipocyte differentiation in 3T3-L1 cells. During adipocyte differentiation, APE1/Ref-1 expression significantly decreased with the increased expression of adipogenic transcription factors such as CCAAT/enhancer binding protein (C/EBP)-a and peroxisome proliferator-activated receptor (PPAR)-g, and the adipocyte differentiation marker adipocyte protein 2 (aP2) in a time-dependent manner. However, APE1/Ref-1 overexpression inhibited C/EBP-a, PPAR-g, and aP2 expression, which was upregulated during adipocyte differentiation. In contrast, silencing APE1/Ref-1 or redox inhibition of APE1/Ref-1 using E3330 increased the mRNA and protein levels of C/EBP-a, PPAR-g, and aP2 during adipocyte differentiation. These results suggest that APE1/Ref-1 inhibits adipocyte differentiation by regulating adipogenic transcription factors, suggesting that APE1/Ref-1 is a potential therapeutic target for regulating adipocyte differentiation.

Keywords: APE1/Ref-1, adipocyte differentiation, adipogenic transcription factor, anti-adipogenic function

Poster

P20-26

Ulinastastin attenuates vascular damage in IDH2-deficient endothelial cells via TGF-β/MMP7/SDS2 signaling pathway

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Syndecan-2 (SDC2), a glycocalyx protein, is highly expressed in endothelial cells and upregulated during inflammation. In our previous study, we have shown that mitochondrial dysfunction in isocitrate dehydrogenase 2 (IDH2)-deficient endothelial cells leads to an increase in inflammation induction. Therefore, we aimed to explore the effect of SDC2 expression in IDH2-deficient endothelial cells. We demonstrated that IDH2 knockdown led to an increase in SDC2, matrix metalloproteinase 7 (MMP7), and TGF-β expression in human umbilical vein endothelial cells (HUVECs). SDS2 level was decreased by MMP7 inhibitor treatment, suggesting that MMP7 affected SDC2 expression via TGF-β pathway in IDH2-deficient HUVECs. Furthermore, Ulinastatin (UTI) reversed the changes induced by IDH2 deficiency in both HUVECs and aorta of mice respectively. These results showed that UTI attenuated vascular endothelial cell damage caused by downregulation of IDH2 via the TGF-β/MMP7 signaling pathway.

Keywords: Ulinastatin, IDH2, SDS2, endothelial cells, vascular damage

P20-27

Amyloid beta oligomer activates the microglial NOD2/RIPK2 signaling via ER stress

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In recent years, the role of neuroinflammation in the pathogenesis of Alzheimer's disease (AD), the most prevalent neurodegenerative disease, has been confirmed. Microglia, the innate immune cells present in the central nervous system (CNS), play a crucial role in the pathogenesis and progression of AD. However, the mechanism by which microglia responds to soluble Amyloid beta1-42 (Aβ) oligomers (1), the most toxic form and cause neuroinflammation in the progression of AD remains unknown. We found that Aβ oligomer-activated microglia showed the upregulation of NOD2 (Nucleotide-binding oligomerization domain 2) and RIPK2 (Receptor Interacting Serine/Threonine Kinase 2) proteins and NOD2/RIPK2/NF-kB signaling activation. Previous study reported that NOD2/RIPK2 signaling links endoplasmic reticulum (ER) stress with inflammation. Activated microglia can release IL-1α, TNFα, and C1q to induce neurotoxic-astrocytes or A1 astrocytes which contribute the death of neurons and oligodendrocytes. Therefore, we hypothesized that Aβ1-42 oligomers can trigger the NOD2/RIPK2 pathway via ER stress and then activate the NF-kB pathway to produce inflammatory cytokines in microglia. Consequently, this could induce neurotoxic reactive astrocytes and cause neuronal cell death. By using synthesized Aβ1-42 oligomers to treat microglia purified from wild-type (WT) mice, we showed that Aβ1-42 oligomers induced an unfolded protein response, subsequently activating the NOD2/RIPK2 pathway. Importantly, our in vivo data suggested that genetic depletion of NOD2/RIPK2 signaling reduces the release of inflammatory cytokines and decreases Aβ plaque pathology, prevents neuronal loss, and improves cognitive behavior in 5xFAD and P301S mice. Our data suggests the detrimental effects of microglia in response to soluble $A\beta_{1-42}$ oligomers, and inhibition of NOD2/RIPK2 signaling in microglia can be a new therapeutic candidate for AD.

Keywords: Alzheimer's disease, amyloid beta oligomer, NOD2-RIPK2 signaling, ER stress, microglia

P20-28

The effect of ginger extract on cisplatin-induced acute anorexia in rats

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Cisplatin is a platinum-based chemotherapeutic agent widely used to treat various cancers. However, several side effects have been reported in treated patients. Among these, acute anorexia is one of the most severe secondary effects. In this study, a single oral administration of 100 or 500 mg/kg ginger extract (GE) significantly alleviated the cisplatin-induced decrease in food intake in rats. However, these body weight and water intake decreases were reversed in the 100 mg/kg

group rats. To elucidate the underlying mechanism of action, serotonin (5-HT) and 5-HT2C, 3A, and 4 receptors in the nodose ganglion of the vagus nerve were investigated. The results showed that cisplatin-induced increases in serotonin levels in both the blood and nodose ganglion tissues were significantly decreased by100 and 500 mg/kg of GE administration. On 5-HT receptors, 5-HT3A and 4, but not 2C receptors, were affected by cisplatin, and GE 100 and 500 mg/kg succeeded in downregulating the evoked upregulated gene of these receptors. Protein expression of 5-HT3A and 4 receptors were also reduced in the 100 mg/kg group. Furthermore, the injection of 5-HT3A, and 4 receptors antagonists (palonestron, 0.1 mg/kg, i.p.; piboserod, 1 mg/kg, i.p., respectively) in cisplatin treated rats prevented the decrease in food intake. Using high-performance liquid chromatography (HPLC) analysis, [6]-gingerol and [6]-shogaol were identified and quantified as the major components of GE, comprising 4.12% and 2.15% of the GE, respectively. Although [6]-gingerol or [6]-shogaol alone failed to alleviate the evoked anorexia, when treated together, the effect was significant on the cisplatin-induced decrease in food intake. These results show that GE can be considered a treatment option to alleviate cisplatin-induced anorexia.

Keywords: chemotherapy-induced anorexia, cisplatin, ginger, nodose ganglion, serotonin

P20-29

Vascular endothelial KLK8 is involved in placental development and fetal growth by regulating spiral artery remodeling

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The placenta plays a central role in pregnancy maintenance, fetal development and maternal adaptive response to pregnancy. Spiral artery (SA) remodeling is the basis for the full development and normal function of the placenta. The aim of this study is to explore the effects of endothelial cell kallikrein-related peptidase 8 (KLK8)on SA remodeling and pregnancy, as well as the underlying molecular mechanisms. It was found that maternal vascular endothelial KLK8 deficiency led to a significant reduction in fetal and placental weight from GD9.5 in mice. Insufficient SA remodeling, inadequate placental blood perfusion and abnormal placental vascular network occurred in maternal vascular endothelial KLK8 deficiency mice. Maternal vascular endothelial KLK8 deficiency did not cause significant changes in populations in neutrophils and macrophages in the decidua. Of note, there was a decrease in the total T cells, but an increase in CD8+ T cells. In addition, the tissue resident NK cells were increased, while the CD49a-Eomes-decidual NK cells were decreased, and naive NK cells were increased, whereas mature NK cells were decreased. Additionally, uNK cells around the SA were significantly decreased in vascular endothelial KLK8 deficiency mice. Transcriptomic analysis of the decidua showed that maternal vascular endothelial KLK8 deficiency results in significant changes in pathways involving cytokine-mediated signaling, monocyte chemotaxis, leukocyte migration, humoral immune response, and production and regulation of vascular endothelial growth factors, etc. In vitro study showed that the conditional media (CM) from increased KLK8 expression in human uterine microvascular endothelial cells (HUtMECs) promoted migration and invasion of HTR8 cells, the extravillous trophoblasts, whereas CM from decreased KLK8 expression in HUtMECs reduced migration and invasion of HTR8 cells. Our study suggested that maternal vascular endothelial KLK8 deficiency leads to insufficient invasion of trophoblasts, impaired SA remodeling, and abnormal placental development, thereby resulting in IUGR. Maternal vascular endothelial KLK8 deficiency leads to a decrease in uNK around the SA during SA remodeling and alterations in the immune microenvironment at the maternal-fetal interface.

Keywords: endothelia, KLK8, placenta, spiral artery

P20-30

Exploring the arousal effect of transcutaneous auricular vagus nerve stimulation: A case series

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The current methods for promoting chronic disorder of consciousness (DOC) include spinal cord electrical stimulation, vagal nerve stimulation (VNS), deep brain stimulation, etc. However, it is difficult to popularize due to high surgical difficulty and cost. Our team has found over the years that the auricular concha is the only area on the surface of mammals where the afferent fibers of vagus nerve are distributed. That is, the auricular branch of vagus nerve is the only neural pathway that can reach the brain by stimulating the vagus nerve on the surface of the body. Our previous study applied transcutaneous auricular vagus nerve stimulation (taVNS) to improve the awareness score of a patient with chronic DOC. To obtain further evidence on clinical efficacy, this study collected 10 subjects with chronic DOC and treated them with taVNS for 7 to 20 days to further explore the efficacy and safety with routine therapy. The revised Coma Recovery Scale (CRS-R) was applied to evaluate the level of consciousness. After treatment, 2 out of 10 subjects showed an improvement of ≥ 3 points in the CRS-R score. For the two significant responders, one patient's hearing improved from no response to being able to repeatedly follow medical instructions, while the other's visual and motor responses improved to being able to track and manipulate objects visually. Another patient's CRS-R score improved by ≥ 2 after treatment. The CRS-R scores of the rest 7 patients remained after treatment. This case series demonstrated that taVNS may have the effect of improving the level of consciousness in patients with chronic DOC.

Keywords: transcutaneous auricular vagus nerve stimulation, chronic disorder of consciousness, arousal promotion, CRS-R rating

P20-31

A cortical circuit mechanism underlying inflammatory chronic pain

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The primary somatosensory (S1) cortex plays a crucial role in receiving and processing various sensory information, including innocuous and noxious stimuli. In the S1 cortex, local circuits are formed by pyramidal neurons and GABAergic neurons, which predominantly consist of somatostatin (SOM), parvalbumin (PV) and vasoactive intestinal peptide (VIP)-expressing interneurons. While alterations in calcium activity of

subtype of S1 neurons comprising the local circuit have been reported in animal models of chronic pain, the emphasis has primarily been on the changes in spontaneous activity. Moreover, the mechanisms that drive these changes remain largely unexplained. In this study, using *in vivo* two-photon calcium imaging, we investigated changes in calcium activity of S1 neurons in response to innocuous and noxious stimuli given to the hind-paw of the mice before and after induction of inflammatory pain, one of the animal model of chronic pain. After inducing inflammatory pain, we observed alterations in the response pattern of a subset of S1 neurons to both innocuous and noxious stimuli. Intriguingly, in a small number of neurons, we found that the responsiveness to innocuous and noxious stimuli were reversed, which is not intuitively understood. In the subsequent experiments, we utilized transgenic mice that expressed a genetically encoded calcium indicator (GCaMP6f) specifically in VIP neurons to monitor the calcium activity of these neurons in response to both innocuous and noxious stimuli. We revealed that VIP neurons exhibit a heightened response to innocuous stimuli compared to noxious stimuli, and we also observed the coexistence of both positive signal and negative signal neurons within the VIP neuron population. These results can be an important key to clarify the mechanism of allodynia, which is often accompanied by chronic pain.

Keywords: chronic pain, cortical circuit, somatosensory cortex, allodynia, two-photon imaging

Poster

P20-32

Single cell transcriptome analyses reveal the roles of B cells in fructose-induced hypertension

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Rationale: While the immune system plays a crucial role in the development of hypertension, the specific contributions of distinct immune cell populations remain incompletely understood. The emergence of single-cell RNA-sequencing (scRNA-seq) technology enables us to analyze the transcriptomes of individual immune cells and to assess the significance of each immune cell type in hypertension development.
Objective: We aimed to investigate the hypothesis that B cells play a crucial role in the development of fructose-induced hypertension.
Methods and Results: Eight-week-old Dahl salt-sensitive (SS) male rats were divided into two groups and given either tap water (TW) or a 20% fructose solution (HFS) for 4 weeks. Systolic blood pressure was measured using the tail-cuff method. ScRNA-seq analysis was performed on lamina propria cells (LPs) and peripheral blood mononuclear cells (PBMCs) obtained from SS rats subjected to either TW or HFS. The HFS treatment induced hypertension in the SS rats. The analysis revealed 27 clusters in LPs and 28 clusters in PBMCs, allowing for the identification and characterization of various immune cell types within each cluster. Specifically, B cells and follicular helper T (Tfh) cells were prominent in LPs, while B cells and M1 macrophages dominated PBMCs in the HFS group. Moreover, the HFS treatment triggered an increase in the number of B cells in both LPs and PBMCs, accompanied by activation of the interferon pathway.
Conclusions: The significant involvement of B cells in intestinal and PBMC responses indicates their pivotal contribution to the development of hypertension. This finding suggests that targeting B cells could be a potential strategy to mitigate high blood pressure in fructose-induced hypertension. Moreover, the simultaneous increase in follicular B cells and Tfh cells in LPs, along with the upregulation of interferon pathway genes in B cells, underscores a potential autoimmune factor contributing to the pathogenesis of fructose-induced hypertension in the intestine.

Keywords: single-cell RNA-sequencing, immunity, hypertension, B cell, interferon pathway

P20-33

Akkermansia muciniphila extracellular vesicles have a protective effect against hypertension

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Akkermansia muciniphila (Am) shows a beneficial role as a probiotic in treating metabolic syndrome. However, the mechanism remains to be elucidated. We tested the hypothesis that Am extracellular vesicles (AmEVs) protect against hypertension. Extracellular vesicles purified from anaerobically cultured Am were characterised by nanoparticle tracking analysis, transmission electron microscopy, and silver stain after sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). AmEVs (1.0×10^{10} log particles/L) or vehicles were added into organ baths to induce vasorelaxation. In addition, AmEVs (1.0×10^{8} or 1.0×10^{9} particles/kg) or vehicles were injected into the tail veins of Wistar-Kyoto rats (WKYs) and spontaneously hypertensive rats (SHRs) weekly for 4 weeks. Peripheral blood mononuclear cells (PBMCs) and splenocytes isolated from both rat strains were analyzed by flow cytometry, RT-qPCR, and western blot. AmEVs affected neither vascular contraction nor endothelial relaxation in thoracic aortas. However, AmEVs increased the population of T regulatory (Treg) cells and tended to reduce proinflammatory cytokines. These results indicate that AmEVs have a protective effect against hypertension without causing adverse reactions. Therefore, it is foreseen that AmEVs may be utilized as a novel therapeutic for the treatment of hypertension.

Keywords: Akkermansia muciniphila, hypertension, extracellular vesicles, Treg cells, spontaneous hypertensive rats

P20-34

Neural responses of VPm thalamus on texture discrimination task

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Mice use active whisking in order to discriminate objects. In sensory discrimination, the level of state strongly influences sensory acuity. VPm thalamus neurons, which lie in the Whisker-related sensory

pathway, can be regulated by astrocytes to modulate their response to stimuli. Activation of thalamic astrocytes is regulated by NE, and this neuromodulator system exhibits state-dependent activity. To find out whether activity level of astrocytes by state change can modulate the discrimination task performance and thalamic representation of various texture discrimination, we developed a closed loop texture discrimination task for head fixed mice to analyze discrimination performances and neuronal activity only in motivated trials — meaning the subjects are involved in discrimination trials only when they intently move forward (i.e. motivated to move). Using textures with various roughness levels, we found that the closed loop task can show discriminatory performance levels. To see the response of the thalamus to various texture stimuli in the no motivated state, We performed the texture discrimination task without reward (Just exploring touch). In this task, thalamus neurons mainly showed 4 subtypes – Activated/inhibited touch units, Activated/inhibited touch units pressure units, and some neurons showed selectivity upon each stimulus.

Keywords: somatosensory, thalamus, discrimination, touch, astrocyte

P20-35

Demystifying molecular basis of sleep: intracellular signaling behind regulation of sleep depth and quantity

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This study is based on our discovery of salt-inducible kinase 3 (SIK3) kinase as a key regulator of sleep homeostasis through the forward genetics study using mice. *Sleepy* splice mutant allele of *Sik3* led to a marked increase in total time and EEG delta power (1-4 Hz) during NREMS. In an independent screening, a second hypersomnic mutant pedigree was discovered with loss-of-function *Sleepy2* splice mutant alleles in *Hdac4*. Next, we generated CRISPR/Cas9-driven gene-modified mice harboring *Hdac4* splice mutation (herein denoted as $Hdac4^{SA}$), which exhibited increased total time and delta power during NREMS, similar to the *Sleepy2* pedigree. In contrast, the phosphor-deficient HDAC4 mice of SIK3-targeted phosphorylation site, $Hdac4^{S245A}$, showed an opposite trend with a decrease in the NREMS time and delta power. Additionally, somatic expression of $Hdac4^{S245A}$ in $Sik3^{Sleepy}$ mice via CNS-transducible adeno-associated virus alleviated the hypersomnic phenotype of $Sik3^{Sleepy}$ mutants, suggesting a direct link between SIK3 and HDAC4 in NREMS regulation.

Furthermore, neural group and cell-type specific manipulations of the kinase-substrate pair using the Cre/loxP recombination system revealed distinct subsets of neurons that differentially regulate NREMS time and delta power. Manipulation of SIK3-HDAC4 in cortical excitatory neurons led to changes in delta power during NREMS, indicating its involvement in regulating sleep depth, whereas manipulation in the hypothalamic excitatory neurons was associated with changes in the NREMS time, or sleep quantity. In addition, we identified a subset of transcripts that were commonly regulated by the expression $Sik3^{Sleepy}$ and sleep deprivation in cortical glutamatergic neurons. These results suggest the common intracellular signaling components and the link between the cellular and circuitry mechanism that control the quality and quantity of NREMS.

The ongoing study continues to investigate the underlying mechanism of SIK3-HDAC4 signaling in sleep homeostasis regulation, including deciphering the role of HDAC5. HDAC4 and HDAC5 belong to the Class IIa histone deacetylase family with approximately 70% similarity. Hdac5 splice mutant mice also showed increased NREMS time and delta power, similar to $Hdac4^{SA}$. Importantly, HDAC5 shares the SIK3 recognition motif around the serine 245 residue of HDAC4. The specific role of HDAC5 in sleep was investigated by both genetic and somatic manipulation of HDAC5. Whether HDAC4 and HDAC5 play a shared role in sleep regulation or have distinct roles in a region-specific and cell type-specific manner will be discussed in detail.

Here we presented the key intracellular signaling components that regulate the quantity and depth of sleep. Understanding the very mechanism of the pathway will ultimately allow us to understand why people sleep, and to elucidate the pathophysiology of sleep disorders and develop therapeutic interventions.

Keywords: sleep homeostasis, Cre/loxP, Sik3, Hdac4, single-nucleus RNA sequencing

P20-36

Changes in neuronal feature selectivity by synaptic plasticity and astrocytic tonic GABA in the ventrobasal (VB) thalamus

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Sensing and distinguishing sensory information is important for survival. Thalamus is known as a sensory station and the central processing center of sensory stimuli. Thalamic astrocytes regulate the discriminability of somatosensory stimuli by modulating neuronal activity through tonic GABA. However, the mechanism by which discriminability is increased in response to ongoing stimuli remains unclear. Here, we show that thalamic neuronal plasticity and tonic GABA from astrocyte are essential for increasing the neuronal feature selectivity. Using ex vivo electrophysiology, we found that the increase of neuronal feature selectivity is dependent on the lemniscal synapse. Further, we unveil that the somatosensory nucleus in the thalamus (VB) is sensitive to stimulus kinetics by reverse correlation. We observed that neuronal plasticity play a crucial role in regulating the sensitivity of neurons to sensory information. In addition, tonic GABA, produced by astrocytes, indicates the potential for involvement in sensory processing by modulating neuronal activity.

Keywords: somatosensory processing, thalamus, tonic GABA, astrocyte

P20-37

Biomaterials regulate the intracellular signaling pathways of cell proliferation

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Biomaterials are a promising material for bone regeneration. Although biomaterials promote cell proliferation, their specific effects on cellular signaling pathways remain unknown. Here, we treated osteoblast cells at two different concentrations to evaluate their effects on cell proliferation, biomineralization, and signaling pathway activation. All biomaterials increased cell proliferation after 5 days, doubling

cell proliferation after 11 days. HA, and β-TCP decreased intracellular calcium level compared OCP, and without treatment. Under the 10 mg/ml treatment, all biomaterials increased p38 phosphorylation but did not influence the total protein level. Under the 4 mg/ml treatment, β-TCP and OCP increased p38 phosphorylation but HA did not. SB203580 inhibited p38 phosphorylation with or without biomaterials and PD98580 inhibited phosphorylated JNK signaling with or without biomaterials; the biomaterials also inhibited JNK phosphorylation. Furthermore, biomaterials treatment decreased the total AKT level, which is a key mediator of cell proliferation, by more than 50%; however, AKT phosphorylation was considerably increased. Wortmannin, an AKT inhibitor, inhibited phosphorylated AKT signaling. Additionally, the biomaterials had no effect on the total level of Raptor, which is related to the AKT signaling pathway; however, they decreased phosphorylation of the epidermal growth factor, which activates numerous cellular signaling pathways. Thus, the biomaterials increased cell proliferation and ALP activity and regulated several intracellular signaling pathways.

Keywords: biomaterials, cell signaling, cell proliferation

Poster

P20-38

Differential contribution of caveolae to serotonergic and adrenergic vasoconstriction in rat arteries

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Caveolae are invaginated, Ω-shaped membrane structures. They are now recognized as portals for signal transduction of multiple chemical and mechanical stimuli. Notably, the contribution of caveolae has been reported to be receptor-specific. However, details of how they differentially contribute to receptor signaling remain unclear. Using isometric tension measurements, patch-clamping, and western blotting, we examined the contribution of caveolae and their related signaling pathways to serotonergic (5-HT_{2A} receptor-mediated) and adrenergic (α1-adrenoceptor-mediated) signaling in rat mesenteric arteries. Disruption of caveolae by methyl-β-cyclodextrin effectively blocked vasoconstriction mediated by the 5-HT_{2A} receptor (5-HT_{2A}R), but not by the α1-adrenoceptor. Caveolar disruption selectively impaired 5-HT_{2A}R-mediated voltage-dependent K^+ channel (Kv) inhibition, but not α1-adrenoceptor-mediated Kv inhibition. In contrast, both serotonergic and α1-adrenergic effects on vasoconstriction, as well as Kv currents, were similarly blocked by the Src tyrosine kinase inhibitor PP_2. However, inhibition of protein kinase C (PKC) by either GO6976 or chelerythrine selectively attenuated the effects mediated by the α1-adrenoceptor, but not by 5-HT_{2A}R. Disruption of caveolae decreased 5-HT_{2A}R-mediated Src phosphorylation, but not α1-adrenoceptor-mediated Src phosphorylation. Finally, the PKC inhibitor GO6976 blocked Src phosphorylation by the α1-adrenoceptor, but not by 5-HT_{2A}R. 5-HT_{2A}R-mediated Kv inhibition and vasoconstriction are dependent on caveolar integrity and Src tyrosine kinase, but not on PKC. In contrast, α1-adrenoceptor-mediated Kv inhibition and vasoconstriction are not dependent on caveolar integrity, but rather on PKC and Src tyrosine kinase. Caveolae-independent PKC is upstream of Src activation for α1-adrenoceptor-mediated Kv inhibition and vasoconstriction.

Keywords: caveolae, 5-HT2A receptor, α1-adrenoceptor, voltage-dependent K^+ channels (Kv), protein kinase-C

P20-39

Neurotoxic reactive astrocytes induce mitochondrial dysfunction-mediated neuronal cell death in neurodegenerative disease by exosomal secretion of Drp1

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Neuroinflammation has been shown to play an important role in the development and progression of *neurodegenerative* diseases such as Parkinson's disease (PD) and *Alzheimer's disease* (*AD*). Astrocytes, one of the key regulators in Neuroinflammation, can be transformed into neurotoxic reactive state in response to neurodegenerative diseases and trigger cell death of neurons. However, the mechanism by which neurotoxic reactive astrocytes kill neuron cells has not been fully understood yet. In this study, we activated microglia by treatment of α-synuclein pre-formed fibril (α-syn PFF) or amyloid-beta oligomer (AβO), and collected the activated microglia conditioned media (MCM). We then incubated astrocytes in activated MCMs to generate neurotoxic reactive astrocyte. SILAC (Stable isotope labeling by amino acids in cell culture) based proteomic analysis of secretomes showed that secreted Drp1 (dynamin-related protein 1) was increased in neurotoxic reactive astrocyte induced by activated microglia. Next, we found that secreted Drp1 from neurotoxic reactive astrocyte was translocated into neuron cell and localized in mitochondria. Additionally, mitochondrial Drp1 was shown to induce mitochondrial dysfunction which sequentially induced cell death of neuron cells. Interestingly, we discovered that Drp1 was released from neurotoxic reactive astrocyte via exosome not exocytosis. When we isolated exosomes from neurotoxic reactive astrocyte and treated them to neuron cell, we also observed the mitochondrial dysfunction of neuron cells with localization of Drp1 on mitochondria and neuronal cell death. In addition, we showed that exosomal Drp1 increased α-synuclein aggregation on α-synuclein induce in-vitro cell model of Parkinson's disease (PD). Taken together, we suggested that Drp1 had critical role in astrocyte-induced neuronal cell death in Parkinson's disease.

Keywords: astrocyte, Parkinson's disease, Drp1, mitochondria, exosome

P20-40

Agomelatine alleviates obesity induced kidney damage through the inhibition of renal inflammation and necroptosis pathways in obese insulin resistant rat model

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Introduction: Chronic kidney disease (CKD) is the most common complication that could occur in obesity. The excessive intake of high-fat food not only elevate body weight gain but also increase adipocyte size and adipocyte hypertrophy. These can stimulate the releasing of proinflammatory cytokines to systemic and contribute to kidney inflammation and injury. Necroptosis is one of the program cell death which is initiated by inflammatory cytokines and eventually lead to

cell membrane breakdown and cell death. Agomelatine (AGOM), a structural analog of melatonin, is a relatively new drug that is prescribed for the management of depressive disorders. Previous study found AGOM improved kidney injury under high-fat diet condition through the inhibition of oxidative stress, ER stress and apoptosis. Impaired autophagy could be reversed after AGOM treatment in kidney cell and protected kidney injury under obese condition. However, the effect of AGOM supplementation on renal inflammation and necroptosis in obese condition has never been elucidated.
Materials and methods: Male Wistar rats were received normal diet (ND) or high-fat diet (HF) for 16 weeks. Then, the HF rats were separated into 3 subgroups including (1) HF; (2) Agomelatine (AGOM), the rats were received AGOM at the dose of 20 mg/kg/day; (3) Pioglitazone (PIO), the rats were received PIO at the dose of 10 mg/kg/day by oral gavage for 4 weeks. After 4 weeks of treatment, all rats were sacrificed. Blood, urine, adipose tissue and kidney tissue were collected for further investigations.
Results: This study demonstrated that HF rats established insulin resistance and kidney dysfunction. The increasing in body weight and adipocyte hypertrophy were observed in HF rats which subsequently led to renal inflammation and renal cell injury. These alterations were related with adipocytes hypertrophy and the rising in inflammatory cytokines such as TNFα, JNK, p-NF-κB, COX2 and IL6. Furthermore, the activation of TNFα and its receptor can stimulate the pathway of necroptosis which linked to renal cell membrane breakdown and renal cell death. AGOM and PIO treatments protected renal injury and renal dysfunction through the inhibition of inflammation and necroptosis. The over expression of TNFα, IL-6 and TLR4 were blunted after agomelatine administration. Additionally, AGOM and PIO inhibited the expression of necroptosis protein as indicated by the reduction of p-RIP3 and p-MLKL which consequently prevented renal cell apoptosis as represented by the decreasing in TUNEL-positive cell. This study found that AGOM exerted the effect in term of glomerular structure protection which was indicated by the restoring of renal tight junction, claudin-1.
Conclusions: These findings indicated that AGOM prevented kidney injury under obese condition via the inhibition of inflammation and necroptosis and subsequently recovering glomerular structure.

Keywords: agomelatine, insulin resistance, inflammation, necroptosis, obesity

P20-41

Effects of human mesenchymal stem cell-derived mitochondria on the old and young donor hearts

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Introduction: Recent study from our research group showed promising evidences that mitochondrial transplantation improved the donor heart function. Here, we aimed to investigate the impacts of human mesenchymal stem cell-derived mitochondria (MSC-MT) on mitochondrial and cardiac function in young (27 weeks) and aged (43 weeks) mice donor hearts.
Methods: MSC-MT (1ug/ul HTK) was infused to mouse donor hearts through coronary circulation and the hearts were in HTK preservation solution containing MSC-MT (1ug/ul). Langendorff perfusion system was used to assess the heart function. Cardiomyocyte viability post-MSC-MT exposure and mitochondrial membrane potential, activities of ATP synthase and citrate synthase, ATP production and biogenesis and mitochondrial dynamics markers were examined.
Results: Fluorescence microscopy imaging demonstrate that incubation of both young and aged donor hearts with MSC-MT over a 9-hour ex vivo period enabled the transportation of MSC-MT into myocardial tissue. Functionally, MSC-MT incubation increased mitochondrial membrane potential, reactive oxygen species (ROS), ATP synthase and citrate synthase activities similarly in isolated mitochondria of both young and old mice hearts. In the Langendorff perfusion system, the heartbeat and the contractility of the aged heart were greater and the viability of cardiomyocytes was improved following MSC-MT exposure in aged hearts. Between young and old mice hearts, mitochondrial biogenesis marker (PGC-1a) and OXPHOS components (complex I, II, V) were significantly increased in elder mice hearts.
Conclusion: Mitochondrial transplantation improves mitochondrial activity in both young and aged mice donor hearts. Greater cardiac functions were observed in aged groups. These findings provide a proof of concept for the application of exogenous mitochondria as enhancers for donor hearts.

Keywords: mitochondria treatment, heart transplantation, HTK, heart preserve

P20-42

HIIT paradigm: Evaluating alterations in hematological and metabolic parameters

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The study titled "HIIT Paradigm: Evaluating Alterations in Hematological and Metabolic Parameters" sought to assess whether High-Intensity Interval Training (HIIT) could induce significant changes in hemoglobin levels, hematocrit, erythrocyte count, and Body Mass Index (BMI) in comparison to a control group. With a strong interest in HIIT's physiological impacts, two groups were established - an exercise-engaging group put through the HIIT regimen and a control group that did not participate in this high-intensity exercise.
Contrary to popular assumptions, the data generated did not unveil any significant differences in the measured parameters between the HIIT and control groups. The resultant findings suggest the potential resilience of these key health markers to this form of exercise, challenging the commonly held belief that HIIT significantly influences these physiological parameters.
However, intriguingly, both groups demonstrated substantial changes in each parameter when analyzed over time - from the pre-test to the post-test stage. This temporal variation infers the existence of other influential factors that affect these health markers over time. Future inquiries are thus necessitated to clarify the breadth of factors inducing these significant temporal changes, thereby providing more precise insight into an individual's physiological progression or variations across similar examination sessions. The study thus highlights HIIT's limited direct impact on these physiological variables, paving the way for more extensive research discerning the contributing factors to these observed temporal transformations.

Keywords: exercise impact, physiological responses, hemoglobin, hematocrite, erythrocyte

Poster

P20-43

Suppression of TGF-β/integrin signaling by klotho prevents transdifferentiation of hepatic stellate cells and liver fibrosis

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Hepatic stellate cells (HSCs) play a pivotal role in liver fibrosis by transforming into myofibroblasts in response to noxious stresses. Transforming growth factor-β (TGF-β) and integrin signaling are known to be involved in this activation process; however, the precise molecular mechanisms remain unclear. In order to elucidate these key regulatory pathways, we used isolated primary HSCs from balb/C mice which spontaneously activate within 7 days when cultured in polystyrene dishes. The increase in expression and secretion of TGF-β during activation of the quiescent HSCs was abrogated by preventing integrin receptor activation. The resulting autocrine TGF-β-driven signaling cascade involved Smad-2/3-ERK1/2-mTOR-NOX4 activation, leading to ROS generation and fibrogenesis. All of these processes were entirely negated by the application of a TGF-β blocking antibody or TGF-β receptor II inhibitors. Extra- or intracellular Ca^{2+} chelation, as well as Ca^{2+} channel blockers, suppressed TGF-β/integrin signaling and fibrotic changes, suggesting the exclusive Ca^{2+} dependence on HSC activation. Notably, α-Klotho, an anti-aging protein, was able to suppress the transdifferentiation of HSCs in vitro by interacting with both integrin (α_v) and TGF-β (type II) receptors, obstructing their activation by TGF-β. In addition, *in vivo* intraperitoneal administration of α-Klotho significantly alleviated liver fibrosis and injury caused by thioacetamide (TAA). Overall, these results shed light on the critical role of α-Klotho in attenuating liver fibrosis by targeting the TGF-β /integrin signaling pathways in HSCs. These findings hold promise for the development of novel therapeutic strategies for liver fibrosis and, potentially, other chronic fibrotic diseases.

Keywords: hepatic stellate cells, fibrosis, TGF-β, integrin receptor, Klotho

P20-44

Effects of homoharringtonine on muscle atrophy and muscle function in Dmd^{mdx} mice

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Skeletal muscle accounts for approximately 40% of the body weight and plays an important role in regulating and maintaining various biological reactions. Duchenne muscular dystrophy (Dmd) is one of the most common progressive muscular dystrophy diseases. It is a genetic disease and there is still no effective treatment. Previous research results showed that homoharringtonine (HHT) significantly increased skeletal muscle mass and function in models of muscle atrophy caused by aging, obesity, or hindlimb immobilization. Therefore, we tried to determine whether skeletal muscle atrophy and function were significantly improved by applying HHT to Dmd^{mdx} mice.
Method: 8-week-old male C57BL/10ScSn-Dmd^{mdx}/J (#001801, Jackson Lab.) mutant mice were randomly divided for two group (PBS vs. HHT). HHT (#1416, Tocris Bio.) or PBS was administered intraperitoneally at a concentration of 10 uM/g three times a week. The total administration period was 20 weeks. To measure muscle function tests, hanging time and grip strength tests were performed at 10 and 20 weeks. After 20 weeks of drug administration, the patient was anesthetized with Evertin and DXA and tissue sampling were performed. The plantaris muscle was prepared with OCT compound for tissue staining, and the rest was clamp-frozen and stored at -80oC until analysis.
Results: The body weight of the HHT group was significantly decreased compared to the PBS group, but there was no significant difference in % body fat and %lean body mass between the two groups. Muscle function in mdx mice was significantly reduced at 20 weeks compared with at 10 weeks in both groups. However, the hanging time of HHT-administered group was significantly higher than that in the PBS group. After 20 weeks of HHT administration, collagen in the plantar muscles of mdx mice was significantly decreased. HHT administration for 20 weeks significantly decreased collagen levels and expression of inflammatory factors in the plantaris muscles of mdx mice.
Conclusion: HHT reduced fibrosis and improved muscle function in mdx mice, which is thought to be due to decreased expression of inflammatory factors in skeletal muscle.

Keywords: homoherrington, duchenne muscular dystrophy, muscle atrophy, inflammation, muscle function

P20-45

Development of ScFv for repression of type II inflammation produced in *nicotiana benthamiana*

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Plant is an attractive expression platform for biologics production system which have benefits in similar glycosylation pattern of mammalian cell protein, low production cost, and no risk from the residual protein or virus infection from host. However, when plant biological products are directly injected into humans, the risk of acute allergic reactions may arise due to plant-specific post-translational modifications, such as plant-specific N- or O-glycosylation, cannot be excluded. For this reason, small antibody fragment ScFv is an ideal production templates for plant biologics because they have outstanding advantages over monoclonal antibody (mAb) such as low molecular weight or high penetration capacity, which provides a non-invasive therapeutic route.
Dupilumab is a humanized IgG4 monoclonal antibody that targets the IL-4 receptor alpha chain (IL-4Rα). Blockade of IL-4/IL-13 by dupilumab is dramatically effective to block Th2 immune response, which triggers a severe allergic inflammation, such as asthma, atopic dermatitis, and rhinitis. However, the reported adverse effects of dupilumab, such as injection site reactions, swelling, and itching, are mainly caused by its injection administration route. In this study, we developed a dupilumab single-chain variable fragment (Dup-ScFv) produced in *Nicotiana benthamiana* not only to increase tissue penetration capacity via decreasing the size, but also to improve the economic aspect of production as well as the preference of host system.
In this poster, we present the design, expression and purification, and production yield of Dup-ScFv in *Nicotiana benthamiana*. Also, the binding affinity of Dup-ScFv via ELISA, IL-4Rα expressing cell binding, as well as SPR is compared with dupilumab. Furthermore, the IL-4 and IL-13 blocking efficacy of Dup-ScFv is analyzed by reporter cell system. Additionally, we have examined western blot and qPCR data

investigating whether Dup-ScFv can exhibit blocking activity by assessing inflammatory response markers in Human Nasal Epithelial Cells. The discussion about the special benefits of plant biologics, especially for non-invasive treatment, will be highly expected.

Keywords: allergic inflammation, ScFv, Dupilumab, plant biologics, non-Invasive

P20-46

The effect of hepatocyte growth factor in TRPV1-mediated pain

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Hepatocyte growth factor (HGF) is a multifunctional factor with a proven importance in survival, as it plays a vital role in the development of organs and in anti-inflammation, repair, and regeneration of damaged tissues. Increased expressions of HGF and its receptor c-MET have been observed in sites of injury followed by recovery which implies HGF is essential in the repair mechanism. The physiological role of HGF in anti-inflammation and recovery after injury has been suggested in many studies, however, how it participates in pain has not been well established. To verify the physiological action of HGF in pain modulation, we observed how application of HGF affects the transient receptor potential vanilloid 1 (TRPV1), the widely known pain receptor. Activation of TRPV1 (both capsacin- and heat-induced pain responses and capsaicin-induced Ca^{2+} increment) has been significantly reduced by recombinant HGF and its naturally occurring variant isoform NK1. In the presence of c-MET inhibitor, PHA665752, neither HGF nor NK1 was able to block the capsaicin-evoked activation of TRPV1 in in-vivo and in-vitro experiments. To further clarify through what pathway HGF blocks the TRPV1, different signaling pathways triggered by HGF have been considered. Inhibitors of corresponding signaling molecules have been tested and results showed only the AKT inhibitor, MK2206-dihydrochloride, was able to rescue the NK1-blocked TRPV1 responses. These findings suggest HGF blocks TRPV1 through the AKT pathway and indicates the physiological importance of HGF in pain, suggesting its pharmacotherapeutic ability in treatment for TRPV1-dependent pain.

Keywords: hepatocyte growth factor (HGF), transient receptor potential vanilloid 1 (TRPV1), pain, channel inhibitor, AKT pathway

P20-47

Interactions between magnesium and calcium ions: Deciphering their impact on pulmonary artery smooth muscle cell calcification

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Objective: To investigate the impact of calcification on pulmonary artery smooth muscle cells (PASMCs), with a focus on changes in magnesium ion (Mg^{2+}) and calcium ion (Ca^{2+}) signaling and energy metabolism.
Methods: (1) A calcification model was established by treating rat PASMCs with 10 mM β-glycerophosphate (β-GP) for 7 days. (2) Calcium deposition was identified through Alizarin Red staining, while the expression of calcification-related factors , Mg^{2+} transport proteins and Canonical transient receptor potential (TRPC) channels was assessed using RT-qPCR and Western blot. (3) Intracellular and mitochondrial Mg^{2+} and Ca^{2+} concentrations were monitored by dynamic fluorescence. Mg^{2+} transport protein channel characteristics and Ca^{2+} influx properties were investigated. (4) The energy metabolism of PASMCs was evaluated by measuring glucose uptake capacity, lactate production, and mitochondrial respiratory function..
Results: After 7 days of high-phosphate culture, PASMCs exhibited significant calcium deposition. The mRNA and protein expression levels of osteogenic markers such as Runx2, Msx2, SOX9, and the pro-calcific factor BMP2 were markedly increased, while the expression of the calcification inhibitor MGP's mRNA and protein was significantly decreased. In comparison to CON-PASMCs, calcified PASMCs exhibited the following changes: (1) Mg^{2+} transporters such as SLC41A1, SLC41A3, and TRPM7 were highly expressed. (2) The resting cytoplasmic free Mg^{2+} concentration ($[Mg^{2+}]_i$) increased, while the mitochondrial free Mg^{2+} concentration ($[Mg^{2+}]_m$) decreased.(3) Increased expression of TRPC1, PRPC4 , and TRPC6, resulting in elevated intracellular calcium concentration $[Ca^{2+}]_i$, enhanced peak values for receptor-operated calcium entry (ROCE) and store-operated calcium entry (SOCE), and a rise in mitochondrial free Ca^{2+} concentration ($[Ca^{2+}]_m$). (4) A decrease in glucose uptake capacity and a reduction in 24-hour lactate production. (5) Increased basal respiration, ATP production, maximum respiration, and spare respiratory capacity.
Conclusion: High phosphate-induced calcification of PASMCs enhances the quantity and functionality of Mg^{2+} transporters, leading to an elevation in $[Mg^{2+}]_i$ and a reduction in $[Mg^{2+}]_m$. Simultaneously, the actions of ROCE and SOCE are augmented, increasing $[Ca^{2+}]_i$ and promoting an elevation in $[Ca^{2+}]_m$. Calcified PASMCs undergo a shift in energy supply, with enhanced mitochondrial oxidative phosphorylation function and a reduced glycolytic level.

Keywords: pulmonary arterial hypertension, vascular calcification, magnesium transporters, TRPC, energy metabolism

P20-48

Role and mechanism of circZKSCAN1 in regulating endothelial cell function and endothelial repair

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Coronary artery disease (CAD) is one of the most common causes of death in the world. The introduction of percutaneous vascular reconstruction has significantly improved the therapeutic effect of CAD patients. However, at the same time, restenosis (ISR) has gradually become the main problem and challenge of PCI treatment CAD. At present, although there are drugs for restenosis, the delivery of these drugs can not completely eradicate ISR, and there is still a risk of intimal hyperplasia, so the exploration of new therapeutic targets and therapeutic schemes for ISR still needs further study. First of all, we make it clear that circZKSCAN1 is a stable ring structure. Fluorescence in situ hybridization and immunofluorescence tests in healthy and restenosis femoral arteries after stenting confirmed that circZKSCAN1 was specifically expressed in the intima of blood vessels with significant differences in expression. In the following in vivo treatment test, we first verified that knocking down circZKSCAN1 could significantly inhibit the proliferation of endothelial cells and expression of necrosis markers RIP3, p-RIP3, p-RIP3, p-MLKL. This is the first time that intimal hyperplasia is related to necroptosis. Next, we explored the molecular mechanism of the function of circZKSCAN1. Through the prediction of biological information website, we found that circZKSCAN1 can encode protein. We designed and constructed an overexpression plasmid with

flag sequence. Western blot detection found that circZKSCAN1 can indeed encode protein. We named the encoded protein circZKSCAN1-203aa. According to the prediction, circZKSCAN1 may encode protein through IRES or m6A. Therefore, we designed IRES mutant, and the luciferase gene report experiment found that IRES was inactive, that is, circZKSCAN1 did not encode protein through IRES. Next, we constructed mutants for the predicted two m6A sites. WB detection found that the expression of flag band was significantly inhibited after the mutation at the 325 site of m6A, that is, circZKSCAN1 translated the protein through the 325 site of m6A. At the same time, the proliferation, migration and necroptosis of HUVECs were also significantly inhibited after the 325-site mutation of m6A. Finally, in order to further regulate the enzyme modified by m6A, we knocked down the translation initiation factor eIF4G2 and the reading protein YTHDF3 of m6A, and found that the coding protein of circZKSCAN1 was significantly reduced, and the proliferation, migration and necroptosis of HUVECs were also inhibited. This result confirmed that eIF4G2 and YTHDF3 are the main regulatory enzymes that regulate the protein encoded by m6A-mediated circZKSCAN1.
Conclusion: CircZKSCAN1 can regulate the damage and repair of vascular intima by affecting the proliferation, migration and necroptosis of HUVECs. In mechanism, the function of circZKSCAN1 is mainly realized through the regulation of protein encoding circZKSCAN1 mediated by m6A modification by eIF4G2 and YTHDF3.

Keywords: circRNA, necroptosis, encoded protein, HUVECs, In-stent restenosis

Poster

P20-49

WITHDRAWN

P20-50

Research on internet-based and real-time drug supervision and regulation in the whole process

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Background: As a commodity of specific importance, drug safety and quality are vital to people's health and lives. Thus, to ensure people's safety and reduce the risk of drug use, the government must formulate regulations and ensure certain interferences to guarantee the quality of drugs while meeting the needs of the people. Recent drug safety incidents and related cases have appeared, large lawsuits with considerable influence have been filed in succession, and the big float led by senior management has been exposed. Illusory advertisements and jumbled drug markets, especially in villages, led to illusory prices and strained physician-patient relationships. Increasing drug supervision and regulation has become a top priority for public safety. Due to the lack of information, such as limited medical information, limited product information, limited manufacturer information, and limited information on consumers, government monitoring of medications and public safety are compromised.
Objective: The objective of this study was to evaluate the methods of drug supervision and regulatory reforms.
Methods: In this study, the literature review, comparative study, situational research, development study, and case study methods were adopted.
Results: The drug industry behaves improperly due to false and asymmetric drug information.
Conclusions: This study is proposed to conduct the Drug supervision and regulation system based on the Internet. The system should consist separate but interconnected real-time updating and dynamic databases, in which drug relavant information should be open and transparent through the system. The system would eliminate the transmission of false drug information and protect the vital interest of the pubilc and defend the good order of the domestic medical industry.

Keywords: drug, internet-based, dynamic database, supervision and regulation, improper behavior

P20-51

Peripheral injection of PNX14 can effectively alleviate chronic heart failure by reducing oxidative stress and inflammation

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ackground and Aims: Chronic heart failure (CHF) is a terminal disease that mainly causes death among patients with cardiovascular diseases. Oxidative stress and inflammation participate in CHF development. Phoenixin (PNX) is a highly secreted peptide widely distributed in the hypothalamus and heart (found in 2013). PNX14 is a highly expressed subtype of PNX in the heart. PNX14 binds to its receptor G protein-coupled receptor 173 (GPR173) during myocardial ischemia for compensatory protection. Exogenous PNX14 can simultaneously reduce heart contractions, induce relaxation, and reduce infarct size. Besides improving systolic function, PNX14 can reduce myocardial ischemia and reperfusion injury.
Methods: In this study, the protective effect of exogenous PNX14 on heart failure (tail vein injection) was investigated in a rat model of chronic heart failure (established by injecting isoproterenol through the abdominal cavity). The effects of PNX14 on inflammation and oxidative stress were also examined.
Results: PNX14 can significantly protect myocardium and alleviate CHF. Furthermore, exogenous PNX14 can effectively inhibit inflammation, alleviate oxidative stress, and reduce myocardial apoptosis.
Conclusions: PNX14 and its receptor GPR173 are potential intervention targets for heart failure treatment.

Keywords: chronic heart failure, PNX14, inflammatory reaction, oxidative stress, receptor GPR173

P20-52

Combination therapy strategy to overcome resistance to EGFR inhibitors in NSCLC with *Paeoniae Radix*

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Non-small-cell lung cancer (NSCLC) accounts for 80-85% of lung cancer cases. As a targeted therapy for NSCLC, epidermal growth factor receptor (EGFR) inhibitors have improved patient survival and quality of life. However, a significant number of patients fail to respond to

treatment with EGFR inhibitors due to drug resistance. Recently, a novel molecular mechanism for the anticancer effects of *Paeoniae Radix* (PR) was identified as inhibition of Aurora B pathway, which contributes to resistance to EGFR inhibitors. In this study, we investigated the synergistic mechanisms of combination treatment with PR to overcome resistance to EGFR inhibitors in NSCLC. The resistance signature gene to EGFR inhibitors was analyzed from expression and drug sensitivity data of cell lines obtained from the DepMap database. And H1650 cells were treated with Erlotinib, Gefitinib and/or PR. And we used RNA-sequencing to examine differential expression between the different treatment groups, including single treatments and combination followed by pathway and function analysis. Compared to the drug-responsive transcriptome of PR, we revealed that PR may suppress the resistance signature by regulating Aurora B pathway as well as p53 and cell cycle pathways. We further found that among the main ingredients of PR, gallic acid, hederagenin, and oleanolic acid were involved in the resistance suppression effect of PR. Also, compared to single treatments and combination transcriptomes of drugs, the p53 pathway, hypoxia pathway, and unfolded protein response were upregulated in combination than single treatments. These results may contribute to the development of natural product-based combination therapeutic strategies to inhibit NSCLC drug resistance.

Keywords: NSCLC, drug resistance, herbal medicine, transcriptome, combination therapy

P20-53

Development of novel agonistic anti-Siglec-8 nanobody for allergic disease therapy

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Mast cells play a central role in the pathogenesis of allergic autoimmune disorders, including allergies, asthma, and atopy. Achieving targeted modulation of immune responses associated with mast cells demands the identification of specific regulatory elements. Siglec-8, an immunoglobulin-like lectin, exhibits a unique affinity for sialic acid, offering a means for precise control over mast cell activity. Nanobodies, compact antibody fragments, provide a platform not only for refined antibody engineering but also for site-specific administration through topical application or spray-based delivery, owing to their compact structure and exceptional stability.

This study aims to regulate mast cell-specific allergic responses through the discovery of a nanobody binding to Siglec-8, an inhibitory receptor exclusive to mast cells and eosinophils. Employing a synthetic nanobody library with a diversity of $1x10^{12}$, we identified 12 nanobodies that bind various regions of Siglec-8, employing ribosomal display, phage display, and ELISA. This presentation will elaborate upon nanobodies capable of activating Siglec-8, thereby mitigating the exacerbation of allergic reactions within mast cells. We will delineate the affinity of each excavated nanobody and its consequential impact on Siglec-8 upon binding to diverse epitopes. This research is anticipated to make significant contributions to the study of mast cell inhibition and maladies originating from aberrant mast cell activity.

This effort represents a significant progress in comprehending the intricacies of inflammatory responses and holds potential for tailored interventions in allergic conditions, particularly atopic dermatitis. By harnessing the therapeutic potential of Siglec-8 and leveraging the distinctive benefits of nanobodies, this innovative approach establishes a sturdy foundation for advancing treatments in conditions marked by dysregulated mast cell function.

Keywords: Siglec-8, nanobody, mast cell, allergic inflammation, Lirentelimab (AK002)

P20-54

Compensatory upregulation and cardioprotective roles of neuregulin-1 in diabetic cardiomyopathy

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Background: Diabetes mellitus is a prevalent risk factor for congestive heart failure. Diabetic cardiomyopathy patients present with left ventricular (LV) diastolic dysfunction at an early stage, then systolic dysfunction as the disease progresses. The mechanism underlying the development of diabetic cardiomyopathy has not yet been fully understood. This study aimed to elucidate the mechanisms by which diastolic dysfunction precedes systolic dysfunction at the early stage of diabetic cardiomyopathy. We hypothesized that the downregulation of cardioprotective factors is involved in the pathogenesis of diabetic cardiomyopathy.

Methods: Animal care and experimental protocols were approved by the Institutional Animal Care and Use Committee of Toho University, and experiments were performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals. Type-1 diabetes mellitus (T1DM) model mice were produced by a single intraperitoneal injection of streptozotocin in 8-week-old male C57BL/6J mice. Age-matched control mice were treated with citrate-buffered saline.

Results: LV diastolic dysfunction, but not systolic dysfunction, was observed in the T1DM model mice 4 weeks after STZ administration (STZ-4W), which mimicked the early stage of diabetic cardiomyopathy. Counter to our expectations, neuregulin-1 (NRG1) expression was markedly upregulated in vascular endothelial cells in the ventricles, the liver, and the kidney in STZ-4W mice. The plasma NRG1 concentration was also significantly increased, suggesting compensatory roles of local and circulating NRG1. To clarify the functional significance of the upregulated NRG1, we blocked its receptor ErbB2 with trastuzumab (TRZ). In STZ-4W mice, TRZ significantly reduced the systolic function without affecting diastolic function and caused a more prominent reduction in Akt phosphorylation levels. In contrast, TRZ did not affect systolic or diastolic function nor Akt phosphorylation levels in the age-matched control mice. A database analysis of a dataset of the left ventricle from heart failure patients revealed that Nrg1 was significantly upregulated in the diabetic group compared to the non-diabetic group. Enrichment analysis showed significant enrichment of the NRG1-ErbB-Akt signaling pathway, suggesting that upregulation of NRG1 in the early stage of diabetic cardiomyopathy may be common in both T1DM and T2DM.

Conclusions: These results indicate that the compensatory upregulated NRG1 contributes to maintaining the LV systolic function, which explains why diastolic dysfunction precedes systolic dysfunction at the early stage of diabetic cardiomyopathy.

Keywords: diabetic cardiomyopathy, neuregulin-1, systolic function, ErbB, trastuzumab

Poster

P20-55

Cerebellar systems consolidation driven by the temporal dynamics of Purkinje cell excitability

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Initially encoded motor memory in the cerebellar cortex becomes mature through systems consolidation. Despite evidence for the involvement of intrinsic excitability of Purkinje cell (PC-IE) in the consolidation of motor memory, the underlying neural mechanism and direct causality between PC-IE and the consolidation await elucidation. Here, we demonstrate the correlation between them through precise temporal profiling of PC-IE during the consolidation period. An optogenetic excitement of PC-IE robustly impaired memory consolidation that is only effective within the 90 min post-learning period, which we defined as an essential time window for memory consolidation. Accordingly, PC-IE becomes depressed shortly after motor learning, but past that critical 90 min post-learning point, the depression is diminished. Furthermore, abnormally increased PC-IE not only disrupts the formation of long-term memory but also abolishes the intrinsic plasticity of flocculus-targeting neurons (FTNs), a post circuitry of floccular PCs, in medial vestibular nuclei (MVN). This finding demonstrates that PC-IE is a neural substrate for coupling the cortex and nuclei, which underlies the systems consolidation. Collectively, these results suggest that the precise timing and temporal dynamics of IE may determine the systems consolidation.

Keywords: cerebellum, Purkinje cells, intrinsic excitability, motor learning, systems consolidation

P20-56

Unraveling the pivotal role of WNK1 in hepatic stellate cell activation and fibrosis

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Background/Aim: Hepatic stellate cells (HSCs) activation is a primary driver of hepatic fibrosis and portal hypertension, often associated with Na^+ retention. WNK (With-No-Lysine [K]) kinases serve as downstream targets of various hormones and growth factors, and their dysregulation has been implicated in HSCs activation. While mutations in WNK1 and WNK4 are known to be associated with ion imbalances, including hyperkalemia and hypertension, the precise involvement of WNK kinases and their downstream effectors in HSC activation and Na^+ retention remains largely unexplored. This study examines the expression and functions of WNK1 and its downstream regulators, including SPAK, OSR1, and NKCC1, in the context of hepatic fibrosis and HSC activation.
Methods: We employed Differential Expression Genes (DEGs) analysis in R, specifically using DESeq2 and Limma, to identify significant changes in WNK expression levels associated with the presence of hepatic fibrosis. To induce hepatic fibrosis, we utilized bile duct ligation (BDL) and thioacetamide (TAA) administered animal models, as well as cultured human and isolated primary HSCs for *in vitro* experiments.
Results: WNK1 was found to be predominantly expressed in the liver and HSCs. DEGs analysis confirmed the upregulation of the *WNK1* gene in hepatic fibrosis conditions. Subsequent experimental studies similarly revealed a significant increase in the expression of WNK1 as well as SPAK, OSR1, and NKCC1 in both fibrotic liver tissue and activated HSCs. Knockdown of WNK1 using siRNA in human HSCs resulted in reduced expression of a-smooth muscle actin (aSMA), while overexpression of WNK1 exacerbated aSMA expression. Functionally, the motility of human HSC was impaired by pretreatment with bumetanide, a NKCC1 inhibitor. Furthermore, TGFb-induced HSC migration was also inhibited by applying bumetanide in human HSCs.
Conclusion: The WNK1-NKCC1 pathway appears to be involved in HSC activation, leading to hepatic fibrosis. These results provide new insights into the pathogenesis of hepatic fibrosis and open up potential avenues for the treatment of liver cirrhosis.

Keywords: WNK1, hepatic fibrosis, HSCs, DEGs

P20-57

Transcriptome-based systematic analysis of the molecular mechanisms of Bojungikki-Tang on immune cell networks

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Immunotherapy with immune checkpoint inhibitors, including anti-PD-1 antibody, has greatly contributed to cancer treatment, leading to an increase in long-term survival cases for cancer patients. However, immunotherapy has relatively low response rates in cancer patients, and combination therapies are being explored to overcome this limitation. Our previous studies have shown that the combination of Bojungikki-Tang (BJIKT) and anti-PD-1 antibody effectively modulates immune function and significantly inhibits tumor growth. In this study, we adopted a systematic approach using drug-induced transcriptome to elucidate the molecular mechanisms of these BJIKT's synergistic effects. We generated large-scale transcriptome data for five immune cell types (T cell, B cell, macrophage, dendritic cell, and natural killer cell) treated with BJIKT, four representative herbs (*Astragalus mongholicus* Bunge, *Atractylodes japonica* Koidzumi, *Glycyrrhiza uralensis* Fisher, and *Panax ginseng* C. A. Meyer) among ten herbs consisting of BJIKT, and combinations of these four herbs. Through differential expression analysis and route concentration analysis, we revealed that BJIKT regulates immunological functions by improving the tumor microenvironment in a reconstructed network for interaction between five immune cell types and cancer. These findings may contribute to the development of herbal medicine-based immunotherapy combination strategies.

Keywords: herbal medicine, transcriptome, immunotherapy, combination therapy, pathway analysis

P20-58

Exposure to chronic hypobaric hypoxia of 450 mmHg affects reproductive hormones, follicular dynamics, autophagy & related signalling pathways in ovaries of adult female rats

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Background: Hypoxia, characterized by insufficient oxygen supply, can result from factors like high altitudes, pulmonary and vascular conditions, or exposure to environmental pollutants. Existing literature suggests that hypoxia may disrupt ovarian function and hormonal balance, affecting follicular cells, corpus luteum regression, and oocyte aging. Additionally, hypoxia can induce autophagy, a cellular process crucial for maintaining homeostasis, which can have dual outcomes, supporting cell survival or leading to cell death. However, the precise role of autophagy during hypoxia in the ovary remains unclear. Our study aims to investigate molecular changes in the ovaries of 6-month-old female rats exposed to simulated high-altitude hypoxia at 450 mmHg, shedding light on the intricate role of autophagy during hypoxia in the ovary.
Methods: In this study, adult female Charles Foster strain rats were exposed to 450 mmHg simulated hypobaric hypoxia for varying durations (6 hours, 5 days, and 10 days) within a specially designed chamber. The change in level of reproductive hormones like LH, FSH, Estradiol and progesterone were studied through ELISA and alterations in ovarian cytoarchitecture was studied by H&E-stained sections. the analysis of gene and protein expression related to autophagy and apoptosis, (LC-3, Lamp-1, Beclin-1, BAX, BCL-2, and Cytochrome C) and their upstream regulators (HIF-1α, BNIP-3, BECN-1, mTOR, ULK-1, AMPK, AKT, PKC-δ, and JNK-1) were done through qRT-PCR and western blot respectively. TEM was used for ultrastructure confirmation of autophagic structures in ovaries.
Results: Prolonged exposure to hypobaric hypoxia at 450 mmHg resulted in an increase in the levels of estrogen, progesterone, and FSH when compared to the control group. These rats exhibited reduced numbers of primordial and growing follicles. The expression of BAX mRNA decreased, while BCL-2 and LAMP-1 increased. Protein levels of LC-3 II/I, Lamp-1, Beclin 1, and BNIP3 were elevated, whereas SQSTM-1 and Cytochrome C levels declined. Both LC-3 and Bax were detected in granulosa and luteal cells, with Bax showing a higher concentration in granulosa cells situated near oocytes. Furthermore, markers for upstream regulators of autophagy, such as HIF-1α, were found to be elevated, while p-AKT, p-mTOR, and p-ULK-1 exhibited decreased levels.
Conclusion: Chronic exposure to 450 mmHg hypobaric hypoxia led to altered hormone levels and ovarian responses, with increased estrogen, progesterone, and FSH, decreased follicle abundance, and changes in expression of autophagy-related markers and regulatory pathways.

Keywords: hypoxia, autophagy, ovary, LC-3, Beclin-1

P20-59

A novel anti-SIRPα nanobody to induce ADCP for enhanced cancer immunotherapy

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The signal regulatory protein α (SIRPα)/CD47 axis has emerged as a critical immune checkpoint specific to myeloid cells, playing a pivotal role in enabling cancer cells to evade macrophage-mediated phagocytosis. SIRPα expression is notably upregulated in the tumor microenvironment, where it engages the "don't eat me" signal. The interaction between CD47 on cancer cells and SIRPα on macrophages leads to the recruitment of Src homology-2 (SH2)-containing tyrosine phosphatase 1 (SHP-1) and SHP-2, subsequently triggering the phosphorylation of immunoreceptor tyrosine-based inhibitory motifs (ITIMs). These upstream signals effectively prevent the accumulation of myosin-IIA, thus impeding the formation of phagocytic synapses and resulting in phagocytosis inhibition.
To address the structure of nanobodies derived from camelid immune system and to ensure diversity in antigen binding, we constructed a synthetic nanobody library of 1012 variants by introducing diverse variations in the complementarity-determining region 3 (CDR3) of a synthetic nanobody frame with three basic scaffolds. We accomplished the selection of anti-SIRPa nanobodies by ribosome, phage display techniques, and ELISA in a series of screening processes.
In this presentation, we will discuss the screening process of these selected nanobodies and their specific binding to SIRPα, as well as their impact on the binding affinity between SIRPα and CD47. We will also investigate the influence of each nanobody on SIRPα activation, as measured through a reporter cell system utilizing NFkB promoter-driven luciferase and FcγR-dependent phagocytosis in THP-1 cells differentiated into macrophages. It can demonstrate our innovative approach to overcome the challenges associated with the camelid immune system's nanobody architecture and highlights the potential of these newly selected anti-SIRPα nanobodies. This development provides a comprehensive insight for advancing immunotherapeutic strategies aimed at enhancing immune-mediated clearance of cancer cells through the disruption of the SIRPα/CD47 immune checkpoint.

Keywords: antibody-dependent cellular phagocytosis, CD47-SIRPα axis, synthetic nanobody library, tumor microenvironment, immunotherapy

P20-60

Design and development of a cost efficient modular mesoscope for brain imaging in rodents

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The development of cost-efficient and modular imaging systems is indispensable for advancing neuroscience research. Our research focuses on addressing this need by introducing a novel, reversible tandem lens mesoscope tailored for brain imaging in rodents. We created a robust and economical imaging system by utilizing off-the-shelf components. The mesoscope we developed delivers high-resolution structural and functional imaging through the integration of cost-effective lenses and a CMOS camera.
With its unique reversible tandem lens configuration, the mesoscope offers two fields of view (FOV), which can be obtained by rearranging the objective and imaging lenses. The larger FOV (CF1) of 12.6 mm x 10.5 mm provides a spatial resolution of 4.92 µm, while the smaller FOV (CF2) at 6 mm x 5 mm offers a resolution of 2.46 µm. We demonstrate the system's efficacy in imaging neuronal calcium activity in the brains of both rats and mice in vivo.

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The tailored components of the mesoscope ensure its compactness, portability, and versatility. This adaptability allows for easy accommodation of various types of samples and sample holders, enabling a wide range of experiments, both in vivo and in vitro. Our custom-built reversible FOV mesoscope offers cost-effectiveness, with a development cost of under US$10,000, while still delivering exceptional performance.
Such modular and cost-effective imaging tools hold great potential for bridging the gap between optics and neuroscience, thereby advancing neuroscience research through accessible and economically prudent imaging solutions.

Keywords: mesoscope, in vivo, field of view, optics, imaging

P20-61

Effect of P2X7 receptor on spontaneous Ca^{2+} oscillation of microglia cells

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Microglial cells play a vital role in maintaining the equilibrium of the central nervous system and responding to a variety of pathological circumstances. An essential aspect of their functioning involves spontaneous fluctuations in calcium ions (Ca^{2+}), a fundamental signaling mechanism that regulates their surveillance activities and immune responses. Among the numerous purinergic receptors expressed within microglia, the P2X7 receptor emerges as a potential modulator of these oscillations. This study focuses on examining the impact of activating the P2X7 receptor on spontaneous Ca^{2+} oscillations in both BV2 and primary microglial cells. Notably, we observed that both the frequency of spontaneous Ca^{2+} oscillations and the expression of the P2X7 gene were elevated upon stimulation with lipopolysaccharide (LPS). Furthermore, primary microglial cells obtained from mice lacking the P2X7 receptor exhibited a statistically significant reduction in spontaneous Ca^{2+} oscillations compared to those derived from wild-type counterparts. Additionally, our findings confirmed a lowered phagocytic activity in primary microglial cells isolated from P2X7 knockout mice upon ATP stimulation. These results prompt a broader exploration of the functional consequences associated with alterations in Ca^{2+} oscillations within microglia, encompassing their roles in inflammatory responses and neuroprotective functions. A comprehensive comprehension of the intricate interplay between P2X7 receptors and Ca^{2+} oscillations in microglial cells may pave the way for innovative therapeutic approaches targeting neuroinflammatory diseases and neurodegenerative disorders.

Keywords: microglia cells, P2X7, calcium oscillations, phagocytosis

P20-62

Activation of TRPV3 is required for keratinocyte differentiation and epidermal barrier formation

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Proper differentiation of keratinocytes is essential for skin barrier function. An elevated [Ca^{2+}] in the epidermal suprabasal layer is required for the differentiation. TRPV3, a member of the transient receptor potential (TRP) channel family, has been highlighted as the Ca^{2+} influx pathway in keratinocytes. However, the functional change of TRPV3 expression in the differentiation of keratinocytes has not been investigated yet. Here, we investigated the role of TRPV3 in normal human epidermal keratinocytes (NHEKs) and epidermal barrier formation in mice. *In vitro* differentiation of NHEKs was induced via a Ca^{2+}-switch protocol. TRPV3 current (I_{TRPV3}) was measured using whole cell patch clamp technique. Along with the increased expression of TRPV3, increases of loricrin (LRC) and keratin 1 (K1), while decreased keratin 14 (K14), were observed by immunoblotting in the differentiated NHEKs. Those changes were prevented by treatment with ruthenium red, a TRPV3 antagonist. However, an application of 2-APB, an activator of TRPV3, decreased the expression of TRPV3, K14, and LRC, while transiently increased K1. In the mice skin underwent tape stripping test, recovery of transepidermal water loss was enhanced by combined application of TRPV3 agonists (2-APB and carvacrol) while not significantly affected by ruthenium red. Taken together, we found the upregulation and the role of TRPV3 in the differentiation of keratinocytes and skin barrier function recovery.

Keywords: TRPV3, keratinocyte differentiation, epidermal barrier formation, ion channels, calcium

P20-63

Effects of long-term cannabinoid modulation on seizures and associated behaviors in a temporal lobe epilepsy mouse model

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Background: The endocannabinoid system (ECS) is recognized for its role in modulating neuronal excitability. Acute cannabinoid agonist administration is known to have anticonvulsant effects, but the consequences of long-term modulation of tone of endocannabinoid system in epilepsy remain poorly understood. In this comprehensive study, we aimed to investigate the effects of long-term cannabinoid modulation on memory and emotional behavior in a well-established mouse model of temporal lobe epilepsy (TLE), a condition characterized by recurrent seizures and EEG abnormalities.
Methods: TLE mouse model was induced by unilateral dorsal hippocampal injection of Kainic acid (20mM/0.1μl) or saline (0.1μl). After surgery, 24 hours video monitoring was used to verify status epilepticus induced by kainic acid, only mice with confirmed status epilepticus (Racine scale stage≥3) were used in the experiment as a TLE mouse model. Subsequently, the TLE mouse model was administered the synthetic cannabinoid receptor agonist WIN55,212-2 or the antagonist AM 251and DMSO for an extended period of three weeks (Sigma Aldrich). During this period, behavioral seizures and brain wave abnormalities were observed through EEG recording and video monitoring, and behavioral tests including open field, elevated plus maze, Y-maze, resident intruder, forced swimming, and sucrose preference tests.
Results: Intrahippocampal kainic acid injection effectively induced brain wave abnormalities including electroclinical seizures and interictal epileptiform discharges, and these abnormalities were consistent with epileptic symptoms seen on simultaneously recorded video monitoring. Cannabinoid agonist tended to decreased the number and total duration of abnormal brain waves while antagonist increase the number and duration of abnormal brain waves. Cannabinoid antagonist also tended to increase the number and severity of epileptic seizures seen on video monitoring.
Conclusions: We observed that stereotaxic intrahippocampal

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injection of Kainate can induce abnormal brain waves and behavioral abnormalities related to epilepsy. In addition, it was observed that cannabinoid modulation through long-term drug administration rather than acute administration tends to cause not only epileptic seizures but also behavioral changes such as related memory and emotions. In particular, cannabinoid antagonists tended to intensify epilepsy-related changes, suggesting that tonic regulation of the endocannabinoid system is involved in controlling epilepsy symptoms.

Keywords: temporal lobe epilepsy, endocannabinoid system, neurohyperexcitability, emotional behavior

P20-64

Decoding the extracellular matrix uncovers cancer-associated fibroblasts specific to histologic subtypes in gastric cancer

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Gastric cancer is a highly heterogeneous disease regarding histologic features, genotypes, and molecular phenotypes. Compared to cancer cells, research on the heterogeneity of GC stroma is still lacking. Here, we demonstrate extracellular matrix(ECM)-centric analysis, examining its association with histologic subtypes and patient prognosis in human gastric cancer (GC). We conducted a quantitative proteomic analysis of decellularized gastric cancer (GC) tissues, revealing distinct tumorous extracellular matrix (ECM) compositions that highlight the intertumoral variability in ECM proteins. Our results show that the poorly cohesive carcinoma not otherwise specified (PCC-NOS) subtype is distinctly identified from other histologic subtypes. By merging ECM proteomics with single-cell RNA sequencing, we identified essential molecular profiles specific to PCC-NOS stroma. These PCC-NOS-specific ECM proteins (PEMs) exhibited a strong association with adipogenic cancer-associated fibroblasts (adiCAFs). Importantly, both PEMs and adiCAF markers were found to correlate with unfavorable prognosis in GC. In summary, our ECM-focused analysis offers a comprehensive insight into the diverse stromal microenvironment of GC, offering valuable insights into connecting molecular and histologic characteristics.

Keywords: tumor microenviornment, ECM, Gastric cancer, cancer-associated fibroblasts

P20-65

Nutritional content and morphometrics of black soldier fly larvae grown on coconut dregs-based substrate

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Black Soldier Fly Larvae (BSFL) is a high-protein alternative feed item. The nutritional composition of BSFL varies based on the BSFL growing media. The purpose of this research is to assess the productivity and nutritional content of BSFL cultivated on various culture substrates. This study was carried out in July 2023 at Bandung City, West Java, Indonesia. The analysis was carried out at the Animal Feed Chemistry Laboratory, Faculty of Animal Husbandry Padjadjaran University, Indonesia. The study employed RAL with five treatments and four replications, totaling 20 units. The treatments include (T1) 100% fermented coconut dregs; (T2) fermented coconut dregs + 25% fruit dregs; (T3) 75% fermented coconut dregs + 25% laying hen manure; (T4) 75% coconut dregs+ 25% fruit dregs; (T5) 75% coconut dregs + 25% laying hen manure. Each treatment contained 120 grams of neonatal BSFL and placed in a polypropylene tray measuring 40 x 30 cm for 23 days. BSFL prepupae are sun-dried for four days and ground into powder. Tests carried out include weight, morphometrics, and proximate analysis (crude protein, crude fat). The results showed that different substrates significantly influenced harvest weight ($P<0.05$). T4 was the most significantly different ($P<0.05$), but not different from T2. Meanwhile, BSFL length was significantly different at T2 ($P<0.05$) compared to T5, but not significantly different from T1, T3, and T4. The best crude protein and crude fat content was T5, with a respective protein content of 27.28% and fat of 16.21%. In conclusion, in terms of production, BSFL cultivation media in the form of coconut pulp waste can be utilized and based on its protein content, it can be used as an alternative to fish meal in poultry feed.

Keywords: BSFL, coconut dregs, proximate, morphometric, weight

P20-66

Neural mechanisms of spatial learning: A comparative study of successor features and predecessor features algorithms

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Predictive map theory, one of the theories explaining spatial learning in animals, is based on successor representation (SR) learning algorithms. In the real world, agents such as animals and robots are subjected to noisy observations, which can lead to suboptimal actions or even failure during learning. In this study, we compared the performance of the Successor Features (SFs) and Predecessor Features (PFs) algorithms, variants of SR learning, in a one-dimensional maze environment with varying levels of noise. Our results demonstrated that PFs learning consistently outperformed SFs learning in terms of cumulative reward and average step length, even in the absence of noise. As the noise level increased, our study revealed that PFs demonstrated higher resilience than SFs, indicated by a smaller degree of deterioration in both cumulative reward and average step length. Our study's findings suggest that PFs learning's superiority in performance could be due to its capacity to transmit temporal difference errors to a greater number of preceding states, which are independent of the reward function. In the discussion section, we discuss the biological mechanisms involved in the use of PFs algorithms for spatial learning. This study adds to the growing body of theoretical research in the field of computational neuroscience using reinforcement learning algorithms, while also showcasing the practical potential of PFs learning in applications such as robotics, game AI, and autonomous vehicle navigation.

Keywords: reinforcement learning, successor feature learning, predecessor feature learning, navigation, noisy environment

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Probiotic composition of fermented cow and soy milk effect to improving enzyme activities and decreasing blood lipid in female wistar rats

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Isoflavone affects countering the progression to improve the intestine and blood lipids, while probiotics boost the absorption of nutrients and maintain blood lipids. Fermentation of isoflavone might be more beneficial for inducing cytokine systems. There needs to be more information about the difference in effect between the various formulation of fermented cow milk and soy milk towards decreasing blood cholesterol and improving enzyme activities. Twenty-five female rats were divided into five groups for 12 weeks of oral gavaging supplementation of a fermented combination of soy and cow milk. Specific probiotics composition was utilized for fermenting cow and soy milk (*Lactobacillus bulgaricus*, *Streptococcus thermophiles*, *Lactobacillus acidophilus*, and *Bifidobacterium* sp.). Our data showed that lipase enzyme activity improves and decreases the blood cholesterol level.
Histology showed that the epidermal skin thickness was higher in every treatment group. Similar results are found in EGF protein levels. Fermented cow milk with *Bifidobacterium bifidum* and *Lactobacillus acidophilus* were found to have a thicker epidermal and higher level of EGF protein level. Milk fermented by Bifidobacterium sp. showed the most significant effect in improving blood lipids. Results indicated that the activity of lipase and blood cholesterol level in milk fermented with a combination of *Lactobacillus acidophilus* and *Bifidobacterium* are 0.45 units/ml and 169.5 mg/dl, compared with the *Lactobacillus bulgaricus* and *Streptococcus thermophilus* milk fermented that is 0.18 units/ml, and 224.3 mg/dl. Therefore, it might improve enzyme activity and blood lipid compared to the other formulations. Compared to conventional fermented milk, soy milk's composition might enhance fermented milk's positive effect on blood lipids.

Keywords: fermented milk, probiotics, isoflavone, blood cholesterol, lipase activity

P20-68

QR-code linked bite-sized teaching videos as a powerful tool to facilitate active learning in the post-corona smartphone era

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Background: Physiology lab classes teach the way how we physiologists think. So, student supervision requires appropriate experimental instructions and clear discussion for the students' results, interpretations, and questions. Therefore, quality lab classes demand a sufficient number of well-trained supervisors; however, that is not always possible, especially when limited human resources are only available. Here, I did overcome this dilemma by using QR-code linked bite-sized teaching videos to deliver experimental instructions and discussion to students' smartphones on demand.
Methods: Students were provided with lab-class instruction booklets with regular texts and figures, and additionally, QR-codes for smartphone scanning. The QR-codes were linked to bite-sized instruction/discussion videos, so that students can access to the teaching videos on demand directly from their smartphones. The QR codes decoded links to a relay web-server, which provides access analysis capability and necessary flexibility in video links. The relay web-server automatically forwarded the students' access requests to a video server having user authentication. User authentication is mandatory to make the video delivery to comply with Japanese copyright law.
Results: In physiology lab classes at Saga University in September 2023, sophomore medical students were provided with the instruction booklets with QR codes. Prior to the lab-class, the students were assigned to underwrite their lab reports in advance, in order to raise their own questions in this flipped arrangement. At the same time, they were allowed to view the QR-code linked bite-sized instruction/discussion videos from home and in the lab on their smartphones. In the actual lab-classes, with intended minimal teachers' supervision, the students smoothly accomplished their lab-tasks and discussed their own questions by themselves, actively watching the QR-code linked videos to answer their own questions. Statistics of post-class survey indicated this video-assisted active learning helped the students' understanding.
Conclusions: QR-code linked videos printed on teaching materials provide a powerful tool to facilitate students' question-based active learning in the post-corona smartphone era.

Keywords: lab class, active learning, QR-code, bite-sized video, post-corona

P20-69

Arrhythmogenesis in heart cells involves reverse E-C coupling and reverse electrotonic conduction along T-tubules

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Early after depolarization (EAD) is an abberant cardiac afterpotential that underlies the development of life-threatening ventricular arrhythmias. It is believed that the development of EAD is caused by the reactivation of L-type Ca^{2+} current during the period of the action potential plateau; however the cellular mechanisms that underlie the development of EAD is still controversial. One favorable alternative is the depolarizing reverse-mode operation of the Na^{+}/Ca^{2+} exchanger, which is activated by aberrant Ca^{2+} release from the sarcoplasmic retuculum in the process of reverse E-C coupling. Since EADs develop preferentially in damaged heart cells with abnormal Ca^{2+}-signaling, here I studied the causal link between the development of EADs and aberrant intracellular Ca^{2+} level ($[Ca^{2+}]_i$) dynamics in mouse heart cells, using the whole-cell clamp technique. My results show: 1) The generation of EADs was preceded by the development of depolarizing membrane potential (V_m) fluctuation. 2) The depolarizing V_m fluctuation is associated with $[Ca^{2+}]_i$ elevation, suggesting an involvement of reverse E-C coupling via the Na^{+}/Ca^{2+} exchanger. 3) Extending the T-tubules' length constant by decreasing the extracellular K^{+} level facilitated the development of the V_m fluctuation and EADs. Taken together, I conclude that EADs are caused by the depolarizing V_m fluctuation, which is induced locally in the T-tubule membrane by aberrant $[Ca^{2+}]_i$ elevation and is conducted back electrotonically along the T-tubules.

Keywords: arrhythmia, patch clamp, T-tubule, reverse E-C coupling, Na/Ca exchanger

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Nicotinic regulation of thalamocortical input-induced feedforward processing in mouse primary auditory cortex

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Systemic nicotine exposure is known to improve sensory cognitive function and attentional behavior in mammals including humans. In the rodent primary sensory system, acute nicotine exposure enhances thalamocortical axon excitability and increase synchronous synaptic inputs to primary auditory cortex (A1). However, how and which cell types of thalamorecipient cells receive nicotinic regulation remains unclear. We used whole-cell patch-clamp recordings and investigated the nicotinic effects on excitatory neurons and inhibitory fast spiking (FS) interneurons in the thalamorecipient layers 3/4 of A1. We prepared auditory thalamocortical (TC) slices, which maintain TC axons from the ventral division of medial geniculate body (MGv) to A1, using adolescent female mice (postnatal days 26-30, C57BL/6J and GAD67-eGFP knock-in mice, ICR.Cg-Gad1 strain). To elicit TC synaptic responses and/or action potentials in patched neurons, a bipolar stimulation electrode was placed on the superior thalamic radiation (STR), the white matter in the auditory TC axon pathway. Neuronal activities in excitatory neurons and FS interneurons were recorded using the whole-cell patch-clamp method. Nicotine was applied locally to A1 or STR via a perfusion needle or in the bath. Nicotine local application reduced TC axon stimulus-evoked action potentials in the excitatory neurons of A1, without affecting TC synaptic inputs nor inhibitory synaptic inputs from FS interneurons. We also observed a reduction in the probability of TC synaptic inputs to FS neurons. Additionally, STR-evoked excitatory and inhibitory synaptic inputs were either increased or decreased by nicotine, depending on cell types, but in such a way to equally shift the synaptic balance towards excitatory dominance. These results suggest that nicotine exposure enhances synaptic inputs in the thalamorecipient layers by increasing the excitatory/inhibitory ratio while reducing intracortical circuit activity. We investigated the nicotinic modulation of TC inputs and subsequent feedforward activities in excitatory neurons and FS cells in the layers 3/4 of A1. While there were little nicotine effects on FS cell-induced IPSCs in excitatory cells, the TC-evoked feedforward inputs to FS cells were reduced in the synaptic release probability. The TC-evoked excitation in excitatory neurons was unexpectedly reduced. However, when analyzing excitatory and inhibitory synaptic inputs separately, nicotine either increased or decreased both excitatory and inhibitory inputs in a cell type specific manner. These results suggest that nicotinic regulation in the thalamorecipient layer depends on cell types or their embedded neuronal circuits.

Keywords: nicotine, primary auditory cortex, thalamocortical system, feedforward processing, inhibitory interneuron

P20-71

Alterations of posture and kinematics of the mouse model of ADHD due to decreased tonic inhibition in the cerebellum

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Attention deficit hyperactivity disorder (ADHD), a neuropsychiatric disorder, usually features memory impairment due to attention deficit, unintended hyperactivity, and impulsivity. However, motor and posture deficits are also reported in ADHD patients. Especially local muscle-using behaviors like using tools (pencil, scissors) and posture instability. Most ADHD studies using animal models mainly focused on the prefrontal cortex (PFC) and striatum for targeting attention and hyperactivity, respectively. In human cases, many clinical imaging showed the involvement of the cerebellum in ADHD. In this study, using GIT1 haploinsufficiency (HE) mice as an ADHD mouse model, we focused on the cerebellum and its functions. We discovered that GIT1 HE mice showed significantly decreased tonic inhibition, impacting the gait posture but not gross motor function. Further, we analyzed the ADHD mouse model behavior using a 3-dimensional (3D) posture estimation tool (AVATAR) and behavior mapping tool (SUBTLE). We found that GIT1 HE mice showed different kinematic features than WT mice. Our study provides the role of the cerebellum in ADHD pathophysiology from gross level to subtle level.

Keywords: ADHD, motor functions, tonic inhibition, cerebellum

P20-72

Minimal burden of somatic mutations leading to epileptogenesis in FCD II

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Brain somatic mutation is a major genetic cause of intractable focal epilepsy such as focal cortical dysplasia (FCD) type II presenting cortical dyslamination and dysmorphic neurons. These somatic mutations, which lead to the hyperactivation of mTOR kinase and causing intractable epilepsy, often present as extreme low level (e.g. less than 1% of variant allele frequency(VAF) or mutational burden) in patient's brain tissues. However, the minimal burden of somatic mutations leading to the dysfunction of entire brain and epileptic seizures remains unexplored. Here, we generate various numbers of mutation-carrying cells ranging from ~1000 to 40,000 in the somato-sensory (SSC) or prefrontal cortex (PFC) of FCD type II mouse models with somatic mutations and examine the relation between mutational burden and epileptogenesis. Interestingly, we found that 8,666 of mutation-carrying cells in SSCx (9,650 in mPFCx), which correspond to 0.02% of variant allele frequency in the hemicortex, was sufficient to cause epileptic seizures. Seizure frequency shows positive but non-linear correlation with the number of mutation-carrying cells. This kind of low level epilepsy threshold was also found in Tsc1 or Tsc2 gene somatic mutation mouse model, with thousands of neurons, or below 0.5% of total hemicortex cell including glia proliferation. Importantly, we also

observed such ultra-low-level of somatic mutation below 0.5% of VAF in a FCD type II patient's brain, which was previously undetected in deep sequencing (>1000X of read depth) of bulky tissues. Therefore, this study suggests that extreme low number of mutation-carrying neurons in the cortex is able to disrupt the function of the entire brain and lead to epileptogenesis in FCD type II.

Keywords: epilepsy, somatic mutation, neural network, focal cortical dysplasia, tuberous sclerosis

P20-73

Differential role of interleukin 1- beta and hepcidin in predicting iron deficiency anemia among IBD patients: a case-control study from Jordan

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Background: Iron deficiency anemia (IDA) is a common extraintestinal manifestation of inflammatory bowel disease (IBD). The aim of this study was to explore the association between interleukin 1- beta (IL-1β) and hepcidin as makers of inflammation in IBD, and hemoglobin (Hb), hematocrit (Hct), ferritin and soluble transferrin receptor as markers of IDA.
Methods: 38 patients with Crohn disease (CD), 32 patients with ulcerative colitis (UC), and 82 healthy controls (HC) were recruited. Medical history was recorded, and serum samples were collected for biochemical analysis.
Results: Prevalence of IDA was (31.6% in CD patients, 50% in UC and 2% in HC). Chi-square test showed a significant association between IBD and anemia (*P*<.001). Multivariate analysis of variance (MANOVA) was used to compare the difference among the 3 group's (HC, CD, UC) composite variable composed of the linear combination of Hb, Hct, IL-1β, and hepcidin. The multivariate test showed a significant overall model (Pillai's trace F-value =2.82, *P*=.003) and the univariate test showed that Hb was the only significant variable [*P*<.005]. The pairwise comparisons demonstrated a significant difference between CD and HC's Hb means (*P*=.039) and between UC and HC's Hb (*P*<.001) and hepcidin (P=.044). Moreover, multivariate linear regression model was used to predict Hb and Hct based on IL-1β and hepcidin levels after controlling for smoking, exercise, morbidities, and iron supplement. None of the inflammatory factors significantly predicted Hct among males. Among females, higher IL-1β levels were associated with lower levels of Hct (*P*=.018) while higher levels of hepcidin were associated with higher levels of Hct (*P*=.01). Moreover, Hepcidin was positively associated with Hb among males (*P*=.003) but was negatively associated with Hb among females (*P*=.017).
Conclusion: This data suggests that inflammatory markers IL-1β and hepcidin may play a distinctive role of development of IDA in IBD patients in a sex-dependent manner.

Keywords: Iron deficiency anemia, Inflammatory bowel disease, hepcidin, interleukin 1- beta, Jordan

P20-74

In Vivo imaging of the angiogenetic process of endothelial progenitor cells in mouse ischemia model

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Research on angiogenesis is an important field for understanding vascular formation and the physiological mechanisms associated with it in the body. Furthermore, it plays a crucial role in researching the prevention and treatment of various diseases. For example, since tumors activate their own nutritional supply by creating new blood vessels, there has been a lot of research in inhibiting angiogenesis. Additionally, understanding the development and treatment of stroke and diabetic vascular necrosis requires angiogenesis research.
To study angiogenesis, hind limb ischemia mouse model is commonly used. This is very useful for studying the role of endothelial cells in blood vessel formation. Therefore, in this experiment, we aimed to use *in vivo* microscope to visualize the process of vascular genesis over time, by injecting Epithelial progenitor cells into the hind limb ischemia mouse model.
To create the hind limb ischemia mouse model, we made an incision in the Balb/c-nu/nu hind limb skin and performed a surgery where we tied two places of the artery with black silk and cut the area between them. We then injected EGFP-tagged endothelial progenitor cells by intramuscular injection. to visualize the existing blood vessels and new blood vessels, we used FSD647-conjugated anti-CD31 antibody to label endothelial cells.
At the 3-day time point after EPC injection, many EGFP-positive blood vessels began to be observed around the ligation site, and some blood vessels were colocalized with CD31 marker signals and EGFP signals. At the 7-day imaging time point, we observed newly formed EGFP-positive blood vessels bypassing the ligation site. At the 14- and 21-day imaging time points, we observed EGFP-positive blood vessels connecting between two suture points.
In this experiment, we observed angiogenesis in the hind limb ischemia mouse model over time using *in vivo* microscopy. The advantage of being able to directly observe the generation and structure of blood vessels *in vivo* at high resolution, as well as reducing artifacts due to individual differences by enabling repetitive imaging within a single individual makes the in vivo microscope an essential technique for blood vessel research.

Keywords: intravital microscope, microscope, in vivo imaging, in vivo, animal modeling

Poster

P20-75

Structural analysis of the multidrug efflux transporter P-glycoprotein bound by an inhibitor

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Multidrug efflux transporters are one of the causes of drug resistance because they reduce drug efficacy by effluxing drugs out of cells. P-glycoprotein (P-gp), one of the ABC transporters, is expressed in various tissues, including the human blood-brain barrier, small intestine, kidney, placenta, and testis. P-gp recognizes a wide range of substrates and can recognize and excrete compounds and peptides with molecular weights ranging from 250 to 4000. Many drugs in the development stage are recognized by P-gp as substrates.

Because P-gp not only loses drug efficacy when released as a substrate but also affects the kinetics of concomitant drugs, the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) require documentation of drug interactions with several transporters, including P-gp, in the approval of new drugs.

However, developing more specific inhibitors is required because P-gp inhibitors inhibit other ABC transporters. Recently, The 3D structures of P-gp bound by third-generation inhibitors have been reported, and the general framework of the inhibitory mechanism has been clarified (Alam A *et al*., Science, 2019; K Nosol *et al*., PNAS, 2020). However, there were some challenges, such as limited resolution and some of the inhibitor density needed to be visible. Therefore, we aimed to determine the structure of inhibitor-bound P-gp to elucidate the binding mode of the inhibitor in more detail. First, we constructed an expression and purification system for human P-gp, referring to previous research papers. A plasmid containing the human P-gp gene was introduced into Expi293F to express P-gp. Human P-gp was then solubilized using a detergent and purified by affinity purification using an anti-His-tag antibody column. The resulting P-gp was then conjugated with an antibody (Fab), and Cryo-electron microscopy observed and measured the sample, and the 3D structure was determined. In this presentation, we will show what we have achieved so far in constructing the expression and purification system for P-gp and analyzing its 3D structure.

Keywords: ABC transporter, P-glycoprotein, structure, membrane protein, cryo-EM

Poster