

P-01-001

Improvement of cognitive impairments in post-menopausal depression via restoration of hippocampal silent synapses with (-)-gallicocatechin gallate-enriched green teaSohyun Kim¹, Sukjin Ko², Ji-woong Ahn², Young-hwan Kim², Ji-hyun Jeong², Seungsoo Chung^{1,2}¹Brain Korea 21 Plus Project for Medical Science, Department of Physiology Yonsei University College of Medicine, Seoul, Republic of Korea, ²BnH Research Co., LTD. Goyang, Republic of Korea

Post-menopausal depression (PMD) is a common psychological disorder, often complicated with neurocognitive impairments. The pathological course is usually progressed by a series of uncontrolled emotional disruptions during menopause. To overcome PMD-induced cognitive deficits, green tea has been suggested as a dietary supplement because of its known effect on improving cognitive dysfunction induced by normal aging or neurodegenerative syndromes. However, its clinical use to improve PMD-accompanied cognitive deficit is still limited due to the controversial role of the active ingredients and ambiguously defined mechanisms of its action. Hereby, we developed modified high-temperature-processed green tea extract (HTP-GTE), which showed lower neuronal toxicity than the conventional green tea extract (GTE). We also demonstrated that HTP-GTE administration prevented the development of learned helplessness (LH) in a rat post-menopausal model. Additionally, HTP-GTE improved LH-induced cognitive impairments simultaneously by rescuing the long-term synaptic plasticity. This occurred via the restoration of silent synapse formation by activating the hippocampal BDNF-tyrosine receptor kinase B pathway in helpless ovariectomized (OVX) rats. Taken together, we also identified that (-)-gallicocatechin gallate was the main contributor to the HTP-GTE effect. Our findings suggested that HTP-GTE has the potential as a preventive nutritional supplement to ameliorate cognitive dysfunctions associated with PMD.

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Keywords: (-)-Gallicocatechin gallate, Hippocampus, Long-term potentiation, Memory, Post-menopausal depression

P-01-002

Inhibitors of angiotensin converting enzyme (ACE) activates the expression of substance P or bradykinin in cultured astrocyte of miceJae-Gyun Choi¹, Dong-Wook Kang¹, Hyun Jin Shin¹, Miae Lee¹, Sheu-Ran Choi², Jung-Mo Hwang³, Cuk-Seong Kim¹, Sang Do Lee¹, Byeong Hwa Jeon¹, Hyun-Woo Kim¹¹Department of Physiology and Medical Science College of Medicine and Brain Research Institute, Chungnam National University, ²Department of Pharmacology Catholic Kwandong University College of Medicine, ³Department of Orthopaedic Surgery Chungnam National University School of Medicine

Angiotensin converting enzyme inhibitor (ACEi) inhibits the enzyme dipeptidyl carboxypeptidase, which is involved in the conversion of angiotensin I to II and degradation of kinins like substance P (SP) and bradykinin (BK). However, the role of ACEi (Captopril and Enalapril) has not yet been studied much about the cultured astrocyte. In light of these observations, we hypothesized that: 1) Involvement of the SP by pretreatment of ACEi (Captopril or Enalapril, 1 or 10 mg/ml) treated with ACE on cultured astrocyte. 2) Involvement of the BK by pretreatment of ACEi (Captopril or Enalapril, 1 or 10 mg/ml) treated with ACE on cultured astrocyte. 3) Protein level of PKC subunits (PKC α , PKC β , and PKC ϵ) of treatment of SP receptor (neurokinin-1 receptor; NK-1R) antagonist (L-733,060, 1 or 3 μ g/ml) and BK receptor (B1R; R 715 or B2R; HOE 140) antagonist (1 or 3 μ g/ml) on the ACE inhibition (24

h) in cultured astrocyte. Astrocyte cultures were prepared from cerebral cortexes of neonatal mice [postnatal day 2 (P2)]. Treatment of the ACEi, for 24 h significantly increased the levels of SP and BK on cultured astrocyte. In addition, suppressed the levels of SP and BK that was induced by pretreatment of ACEi treated with ACE on cultured astrocyte. Only administration of the NK-1R antagonist, L-733,060 reduced the protein levels of PKC β subunit that was induced by pretreatment of ACEi. These indicated that inhibition of ACE increases the levels of SP and BK on cultured astrocyte. Moreover, inhibition of ACE increases the protein levels of PKC β subunit on cultured astrocyte, which stimulate NK-1R signaling, ultimately astrocyte may modulate cell signaling pathway via the mediation of PKC protein levels by ACE inhibition.

Keywords: Angiotensin converting enzyme, Astrocyte, Bradykinin, PKC, Substance P

P-01-003

Generation of an optimized autaptic culture system for studying synaptic functions in autonomic gangliaSeong Jun Kang¹, Choong-Ku Lee², Huu Son Nguyen¹, Jeong Seop Rhee², Seong-Woo Jeong¹¹Department of Physiology Yonsei University Wonju College of Medicine, Wonju, Republic of Korea, ²Department of Molecular Neurobiology Max plank Institute for Multidisciplinary Sciences, Göttingen, Germany

Autaptic synapse called "autapse" is a self-synapse that is a functional connection between a neuron and itself, and is found to be experimentally valuable for studying synaptic transmission and plasticity under physiological and pathological states. Compared with the central nervous system, the molecular and cellular mechanisms underlying the synaptic functions in peripheral autonomic ganglia have been poorly elucidated. Most preceding studies on the autonomic synaptic functions rely on spontaneous connections among multiple neurons in culture. Recently, a few studies on autonomic transmission have employed the autaptic culture formed on non-uniformly sprayed microislands of growth-permissive substrate. Taken together, it is unlikely that all these approaches give consistent and reproducible data collection. The main goal of the present study was to generate an optimized autaptic culture system for studying the synaptic functions in autonomic ganglia. In this regard, firstly, we refined the microislands on agarose-coated culture dish or coverslips using a stamp with arrays of uniform sized microdots. Then, the sympathetic neurons were enzymatically dissociated from the superior cervical ganglia of neonatal rats (P0-P2), and plated onto the stamped culture dishes or coverslips. Nerve growth factor and ciliary neurotrophic factor were supplemented to the culture media for a long-term culture and induction of cholinergic synapses, respectively. Hexamethonium-sensitive fast excitatory postsynaptic currents (EPSCs) were first observed at DIV4. The EPSCs and the readily releasable pool of vesicles increased time-dependently and reached the maximum size at DIV14. Based on these observations, we optimized the culture conditions for reliably developing cholinergic autapses by testing different media, sera, and glial feeder-layers. In conclusion, our optimized autaptic culture system may facilitate the researches on autonomic synaptic functions.

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Keywords: Autonomic ganglia, Autaptic culture system, Autapse, Cholinergic, Synaptic functions

P-01-004

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.

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Keywords: Itch, Pain, Primary somatosensory cortex, Two-photon imaging

P-01-005

Inwardly rectifying potassium channel, Kir4.1 mediates Ca^{2+} entry in the satellite glial cells of sympathetic ganglia under a hypokalemic condition

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In the autonomic ganglia, satellite glial cells (SGCs) envelope the cell bodies of the principal neurons with a narrow gap of about 20 nm, and may play a pivotal role in regulation of the microenvironment surrounding the neurons. Previous studies have shown that inwardly rectifying K^+ channel, Kir4.1, highly expressed in the astrocytes, is critical for extracellular K^+ buffering during excitation of neurons, which may not only protect neurons but also regulate cell excitability. In our preliminary experiments, the Kir4.1 channels were found to express age-dependently in the autonomic SGCs, suggesting the same roles as those in the astrocytes have. Meanwhile, a certain hypokalemic condition was found to cause Ca^{2+} entry into the astrocytes via the Kir4.1 channels although its functional consequence remains unknown. In the present study, we examined whether Kir4.1 channels mediate the Ca^{2+} signaling in the autonomic SGCs during the hypokalemic condition. In this regard, superior cervical ganglia (SCG) were partially digested to produce the isolated neurons attached with the SGCs. Ca^{2+} imaging analysis revealed that the SGCs responded to bath application of low K^+ (<1 mM) solution with a large cytosolic Ca^{2+} transient, while the SCG neurons did not. Removal of external Ca^{2+} made the SGCs to be insensitive

to the hypokalemic condition. Importantly, Ba^{2+} (0.1 mM) reversibly blocked the Ca^{2+} responses of the SGCs. In addition, HEK293 cells were transfected with Myc-Kir4.1(+) vector. Low extracellular K^+ evoked Ba^{2+} -sensitive Ca^{2+} transient in the HEK293 cells transiently expressing the Kir4.1 channels. Taken together, these data suggest that the Kir4.1 channels mediate the low external K^+ -induced Ca^{2+} entry in the SGCs. At present, we are testing the hypothesis that the Kir4.1 channel-mediated Ca^{2+} entry evokes release of active molecules from the SGCs for increasing cell excitability, and thereby the K^+ level around the neurons in the autonomic ganglia.

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Keywords: Satellite glial cell, Autonomic, Sympathetic ganglia, Kir 4.1, Calcium signaling, Hypokalemic

P-01-006

Analgesic effect of intermittent fasting-related orexin A pathway on the formalin-induced acute pain.

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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 the programmed or learned stressful factor can produce antinociception without stress responses because predictable stress may elicit a minimized 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 and 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. Except for 6 hours of AF, co-expression of LH OXA and fos-B (a neuronal activation marker) was increased both in AF and IF groups. The CORT level was increased in 12 and 24 hours of AF, but not 6 and 12 hours of IF. 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: Fasting, Orexin A, Corticosterone, Antinociception, Formalin

P-01-007

Porphyromonas gingivalis directly interacts with nociceptive sensory neurons to produce analgesic effects in chronic inflammatory pain condition

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Periodontitis is a chronic inflammatory disease of the periodontium that

leads to tissue destruction, bleeding, and bone resorption. However, pain, one of the cardinal signs of inflammation, is not commonly accompanied in periodontitis, which is unlike other inflammatory conditions. Although a lack of pain symptoms may contribute to delayed detection and treatment of periodontitis in patients, the cause and mechanism of decreased pain hypersensitivity in periodontitis remain elusive. Given that *Porphyromonas gingivalis* (Pg) is known as one of the keystone pathogens of periodontitis, Pg could be associated with decreased pain hypersensitivity in chronic periodontitis. Thus, this study was aimed to determine whether Pg produces analgesic effects in inflammatory conditions and to examine the underlying mechanisms in nociceptive sensory neurons. Pain-like behavior tests in adult mice were conducted in naïve or complete Freund's adjuvant (CFA)-induced chronic inflammatory pain model after intraplantar injection of Pg. Pg infection in naïve mice did not produce spontaneous pain-like behaviors or thermal and mechanical hypersensitivities, whereas Pg infection significantly increased the number of inflammatory cells in the hind paw. However, Pg infection significantly reversed thermal and mechanical pain hypersensitivities in the CFA model with no loss of intra-epidermal nerve fiber density, indicating a negative correlation between analgesic effects by Pg infection and alteration of nerve innervation. The analgesic effects by live Pg were not recapitulated by heat-killed, gingipain-null mutant (KDP136), or outer membrane vesicles isolated from wildtype or KDP136 in the CFA model. Immunocytochemical analysis revealed co-localization of Pg and DRG neurons, suggesting direct physical interaction between Pg and DRG neurons, but live Pg application did not induce calcium response in DRG neurons. However, calcitonin gene-related peptide (CGRP) level was significantly decreased in the culture supernatant of DRG neurons with Pg. Our results demonstrate that the infection by live Pg with gingipain activity produces analgesic effects in inflammatory conditions, and the decrease in CGRP release from DRG neurons by live Pg suggests the potential contribution of reduced neurogenic inflammation to the Pg-induced analgesic effects. The detailed underlying molecular mechanism for the regulation of neurogenic inflammation by Pg is currently under investigation.

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Keywords: *Porphyromonas gingivalis*, Periodontitis, Inflammation, Pain, Sensory neuron

P-01-008

Effect of exercise on the reserpine-induced pain and depression-like responses via the modulation of brain-derived neurotrophic factor expression in mice

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Pain associated with depression-like symptoms is one of the reasons people seek medical assistance. Various types of pain act as a trigger for depression and about 35% of chronic pain patients suffered from this. Brain-derived neurotrophic factor (BDNF) is one of the well-known neurotrophins that is widely expressed both in the peripheral and central nervous systems. BDNF is involved not only in neuronal growth but also in neuroplasticity. This study was designed to investigate the effect of exercise and the role of BDNF on the reserpine-induced pain and depression-like responses in mice. In order to induce the pain and depression-like behavior in mice, reserpine (RSP, 1 mg/kg) was administered subcutaneously once a day for 3. Exercise was performed by rota-rod tester after RSP injection for 7 consecutive days. Pain behavior response was measured using von Frey filaments both in hind paws and depression-like behaviors were measured by forced swimming and open field test. In addition, immunofluorescence staining was performed on the dorsal root ganglions (DRG) and spinal cord to measure changes of peripheral and central BDNF. Repetitive injection of RSP evoked mechanical hypersensitivity, increased immobility time in the forced swim-

ming test, and reduced moving distance in the open field test. Moreover, in the immunofluorescence results, BDNF was significantly increased by RSP injections in DRG and spinal dorsal area. Exercise significantly attenuated the RSP-induced abnormal sensory response and depression-like behaviors. Additionally, it suppressed the enhanced expression of BDNF in DRG and spinal dorsal area. Repetitive exercise might be an effective and non-invasive option for treating patients suffering from both pain with depression via regulating the expression of BDNF.

Keywords: Analgesia, Exercise, Brain-derived neurotrophic factor, Fibromyalgia, Reserpine

P-01-009

Electrical stimulation of the insular cortex attenuates neuropathic pain via modulation of synaptic plasticity

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Neuropathic pain is an abnormal phenomenon including allodynia or hyperalgesia that persists for days or years. Several studies have reported that the reinforcement of synaptic plasticity deteriorated the symptoms. One of the brain regions related to pain perception and generation of synaptic plasticity is the insular cortex (IC), and many researches have shown that the IC is a potential target to control pain effectively. Although many treatments have been developed to attenuate the neuropathic pain, the side effects and tolerance remained. For patients who are refractory to medical therapies, many prospective cases of brain stimulation in neuropathic pain have reported the pain-relieving effect. However, the fundamental mechanisms of brain stimulation, especially the insular cortex stimulation (ICS), are still elucidated. The aim of this study was to investigate the mechanisms of pain-relieving effect induced by ICS in neuropathic rats. Behavioral tests were conducted to observe the pain-relieving effects in neuropathic rats. Western blot was performed to identify the changes in the expression levels of phosphorylated extracellular signal-regulated kinase synaptic plasticity-related receptors such as the subunit of N-methyl-D-aspartate receptor (NMDAR), and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) after ICS was applied. Proteomics was conducted to detect specific protein expression patterns among groups. Consequently, neuropathic pain was attenuated by ICS and the effect was maintained for 4 days after ICS was stopped. The expression level of NR2A which is a subunit of NMDAR was not changed. However, the expression level of NR2B was decreased after ICS. The expression level of AMPAR also decreased after ICS was applied. Bioinformatics analysis of proteomics suggested that the levels of proteins involved in collapsin response mediator protein 2 (CRMP2) were dramatically altered between groups. These results inferred that the ICS reduced the long-term potentiation (LTP) and CRMP2 could attenuate LTP accompanied by neuropathic pain following neural injury.

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Keywords: Neuropathic pain, Synaptic plasticity, Insular cortex, Insular cortex stimulation, Collapsin response mediator protein 2

P-01-010

Inhibition of Nav1.7 channels in the trigeminal ganglion alleviates pulpitis-induced pain in rats

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Dental pulpitis causes abnormal pain signaling in the peripheral nervous system (PNS), result in ectopic persistent pain and hyperalgesia. However, the Nav1.7 expression related to the change of neuroactivity following pulpitis has not been investigated in the trigeminal ganglion (TG). The aim of our study was to whether the expression of Nav1.7 changes with allyliso-thiocyanate (AITC)-induced pulpitis in the PNS and the neuronal activities of the TGs can be affected by inhibition of Nav1.7 channels. Acute pulpitis was induced through allyliso-thiocyanate (AITC) application to the rat maxillary molar tooth pulp. Three days after AITC application, abnormal pain behaviors were recorded, and the rats were euthanized to allow for immunohistochemical, optical imaging, and western blot analyses of the Nav1.7 expression in the TGs. A significant increase in AITC-induced pain-like behaviors and histological evidence of pulpitis were observed. In addition, histological and western blot data showed that Nav1.7 expressions in the TGs were significantly higher in the AITC group than in the naive and saline group rats. Optical imaging showed that the AITC group showed higher neuronal activity after electrical stimulation of the TGs. Additionally, treatment of ProTxII, selective Nav1.7 blocker, on to the TGs in the AITC group effectively suppressed the hyperpolarized activity after electrical stimulation. These findings indicate that the inhibition of the Nav1.7 channel could modulate nociceptive signal processing in the TG following pulp inflammation.

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Keywords: Nav1.7 channel, Pulpitis, Pain, Trigeminal ganglion

P-01-011

Long-axon adjacent local lymphadenopathy is responsible for vincristine-induced pain via mediating infiltration of the CXCL13+ CX3CR1+ macrophage into the sciatic nerve

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Chemotherapy-induced peripheral neuropathy (CIPN), which frequently occurs in anti-cancer drug treated patients, manifest 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, we 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, we 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, we 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 IL-21 and CXCL13 in sciatic nerve after vincristine treatment. Immunohistochemical analysis revealed IL-21 and CXCL13 are mainly expressed on CD4+ T cells and CX3CR1+ macrophages respectively in the sciatic nerves, while the depletions of NK cells reduced infiltration of IL-21+ CD4+ T cells and CXCL13+ CX3CR1+ macrophages. Additionally, in vivo administration of anti-CXCL13 antibody containing matrigel around the sciatic nerve prevented the development of mechanical pain after vincristine treatment in dose dependent manner. In conclusion, our findings suggest the local immune activation in the long-axon adjacent lymph node is responsible for vincristine-induced pain development via promoting infiltration of CX3CR1+ macrophage with pain generating molecule, CXCL13, in the sciatic nerve.

Keywords: CIPN, Neuro-immune interaction, Lymph node, CX3CR1+ macrophage, CXCL13

P-01-012

Modulation of neuropathic pain through regulation of glial cells in the insular cortex

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The insular cortex (IC) is essential for regulating nociception and is associated with pain behavior. However, analgesic effects of glial inhibition in the IC have not yet been explored. The aim of this study was to investigate pain-relieving effects after glial inhibition in the IC during the early or late phase of pain development. The effects of glial inhibitors in early or late phase of neuropathic pain were characterized in astrocytes and microglia expressions in the IC of an animal model of neuropathic pain. Changes in withdrawal responses during different stages of inhibition were compared and morphological changes in glial cells with purinergic receptor expressions were analyzed. Inhibition of glial cells had an analgesic effect that persisted even after drug withdrawal. Both GFAP and CD11b/c expressions were decreased after injection of glial inhibitors. Morphological alterations of astrocytes and microglia were observed with expression changes of purinergic receptors. These findings indicate that inhibition of glial activity in the IC alleviates chronic pain, and that purinergic receptors in glial cells are closely related to chronic pain development.

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Keywords: Insular cortex, Neuropathic pain, Astrocytes, Microglia

P-01-013

Clemastine attenuates paclitaxel-induced neuropathic pain by improving myelin repair in the sciatic nerveHeejin Jeong¹, Kyeongmin Kim¹, Guanghai Nan^{1,2}, Leejeong Kim^{1,2}, Myeounghoon Cha¹, Bae Hwan Lee^{1,2}¹Department of Physiology Yonsei University College of Medicine, Seoul, Republic of Korea, ²Brain Korea 21 PLUS Project for Medical Science Yonsei University College of Medicine, Seoul, Republic of Korea

Chemotherapy-induced neuropathic pain (CINP) is a common and severe side effect of anti-tumor agents. Impairment of peripheral nerve by chemotherapeutic drugs, including taxanes and platinum derivatives, has been considered to be a major cause of CINP. However, no treatment drugs are currently available. Recent studies have shown that clemastine, a first-generation of antihistamine drug which has been used to treat allergic conditions, enhances remyelination and rescues functional changes in demyelinating brain disease model such as amyotrophic lateral sclerosis. However, it is still unknown whether clemastine can treat CINP. Here, we investigated whether intraperitoneal injection of clemastine could be effective in alleviating paclitaxel (PTX)-induced neuropathic pain and elucidated the underlying molecular mechanisms.

Male mice were injected with PTX for four alternate days, followed by treatment of clemastine for 14 days. PTX induced increased sensitivity to mechanical stimulation as measured using the von Frey test. Compared to the PTX-injected group, mechanical allodynia was significantly reduced in the clemastine-injected group. To examine if clemastine could promote myelin repair in the sciatic nerve, we performed immunohistochemistry with an antibody against myelin basic protein (MBP). The expression of MBP was decreased in PTX-injected groups compared to vehicle-injected group, whereas its expression was significantly increased after clemastine injection, showing that clemastine promotes nerve remyelination in the sciatic nerve of CINP mice. In addition, we examined glabrous skin of the hind paw to compare the degree of nerve ending degeneration in mice at post-injection 21 day. Immunostaining for PGP9.5 revealed nerve ending degeneration in the PTX-injected mice compared to vehicle-injected mice. Notably, in the clemastine-injected group, PGP9.5 immunoreactivity was strikingly increased, showing that clemastine protected against PTX-induced peripheral nerve degeneration. Taken together, our data suggest that clemastine may be a potential therapy for CINP that acts by protecting against nerve degeneration and promoting myelin repair.

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Keywords: Paclitaxel, Clemastine, CINP, Pain

P-01-014

A semi-automated cell counting method for TH-positive dopaminergic neurons in a mouse model of Parkinson's disease using convolutional neural networksMyeong Seong Bak¹, Doyun Kim², Haney Park¹, In Seon Baek³, Sora Ahn⁴, Hi-Joon Park⁴, Sun Kwang Kim^{1,2,3}¹Neurogrin Inc. Seoul, Korea, ²Department of Physiology College of Korean Medicine, Kyung Hee University, Seoul, Korea, ³Department of Science in Korean Medicine Graduate School, Kyung Hee University, Seoul, Korea, ⁴Acupuncture & Meridian Science Research Center Kyung Hee University, Seoul, Korea

Quantification of tyrosine hydroxylase (TH)-positive neurons is essential for verification of Parkinson's disease (PD) modeling in preclinical studies. However, manual scoring of immunohistochemistry (IHC) images is labor-intensive and has less reproducibility due to a lack of objectivity. Several

automated analysis methods for IHC images have been suggested, but they have limitations including low accuracy and difficulty to use in practice. Here, we developed a convolutional neural networks-based deep learning algorithm for TH+ cell counting. Our analysis tool showed higher accuracy than conventional analyses and was feasible to various experimental conditions, such as staining intensity, brightness, and contrast of images. Our semi-automated cell counting algorithm is available for free and provides a user-friendly graphical user interface to help practical applications. In addition, we are developing a web-based graphical user interface (GUI) for easy accessibility and updates of the deep learning model. Together, we expect that this TH+ cell counting tool will contribute to the facilitation of preclinical PD research by saving time and providing objective analysis of IHC images.

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Keywords: Parkinson's disease, Mice, Dopaminergic neurons, Deep learning, Convolutional neural networks, Cell counting

P-01-015

mGluR5-mediated deactivation of mPFC in the neuropathic pain miceMirae Jang^{1,2}, Sang Jeong Kim^{1,2}¹Department of Physiology Seoul National University College of Medicine, Seoul, Korea, ²Department of Biomedical Sciences Seoul National University College of Medicine, Seoul, Korea

The increment of metabotropic glutamate receptor5 (mGluR5) was recognized as an important biomarker for chronic pain and it is generally known to promote activation of Gq-coupled proteins. Chronic pain deactivates the medial prefrontal cortex (mPFC) despite pain-induced upregulation of mGluR5. Thus, previous studies seem to be contradicting each other due to the lack of explanation for the underlying mechanism. We hypothesized that mGluR5 increased mainly in the inhibitory interneuron. Through electrophysiological experiments, we found that mGluR5 agonist (CHPG) increased inhibitory synaptic input to pyramidal neurons in the prelimbic cortex layer 5. In the same context, neuropathic pain mice also increased inhibitory input to pyramidal neurons and it was rescued with the mGluR5 antagonist (MTEP). Furthermore, pharmacological blockade of upregulation mGluR5 using MTEP in the prelimbic ameliorated pain threshold of chronic pain model mice. Increased excitability of parvalbumin (PV) interneuron in the neuropathic pain group was not decreased by MTEP. Also, this increased excitability was diminished in existing synaptic blockers. Together, our data reveal how mGluR5 upregulation results in the deactivation of mPFC in chronic pain states and prove that mGluR5 changed in non-PV interneurons.

Acknowledgement: NRF- 2018R1A5A2025964.

Keywords: MGLuR5, MPFC, Chronic pain, Interneuron, Electrophysiology

P-01-016

Real-time decoding of spontaneous pain from two-photon microscopy images of brain cellular calcium using deep learning

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Chronic pain remains intractable in millions of patients worldwide. Spontaneous ongoing pain is a major clinical problem of chronic pain and extremely challenging to diagnose and treat compared to stimulus-evoked pain. Although extensive efforts have been made in preclinical studies, there still exists a mismatch in focused pain type between animals and human (i.e. evoked vs. spontaneous), which obstructs the translation of knowledge from preclinical animal models into objective diagnosis and effective new treatments. Here, we developed a deep learning algorithm, named AI-bRNN (Average training, Individual test-bidirectional Recurrent Neural Network), to decode spontaneous pain from brain cellular Ca^{2+} signals recorded by two-photon microscopy imaging in awake, head-fixed mice. AI-bRNN robustly determines the intensity and time point of spontaneous pain even in chronic pain models, and evaluates the efficacy of analgesics in real time. Furthermore, AI-bRNN could be applied to various cell types (neurons and glia), brain areas (cerebral cortex and cerebellum) and somatosensations (itch and pain), proving its versatile performance. These results suggest that our approach offer a clinically relevant, quantitative and real-time preclinical evaluation platform for pain medicine, thereby accelerating the development of new methods for diagnosing and treating human chronic pain patients.

Acknowledgement: This work was supported by the National Research Foundation of Korea(NRF) grant funded by the Korea government(MSIT) (No. 2022R1F1A1072901).

Keywords: Two-photon, Spontaneous pain, Primary somatosensory cortex, Deep learning, Brain calcium

P-01-017

Investigation of *C. elegans* learning and memory regulation mechanism by Mitochondrial Calcium Uniporter (MCU-1)

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Calcium enters the mitochondria through the mitochondrial calcium uniporter (MCU), where, in addition to buffering cytosolic calcium, it regulates important cellular processes such as ATP synthesis and apoptosis. In the neuron, calcium is involved in many aspects of neuronal function, such as neurotransmitter release and plasticity. However, few studies exist that explore MCU's function in neurons and learning and memory. Most of the existing studies of MCU in learning and memory focus on degenerative conditions, where functional downregulation of MCU is beneficial, as it prevents mitochondrial calcium overload and thus neuronal degeneration. Using *C. elegans*, we found that aversive olfactory learning was defective in *mcu-1* mutants, the *C. elegans* ortholog of MCU. Using transgenic strains and pharmacological inhibitors, we found that *mcu-1* was required in the sensory neuron, at the time of learning. Interestingly, overexpression of *mcu-1* resulted in the opposite phenotype: worms showed prolonged memory retention. In addition, the *mcu-1* mutant also had abnormalities in salt learning, suggesting that MCU-1 is involved in other types of memory. We are currently trying to understand how mitochondrial calcium affects learning and memory formation, and memory decay.

Keywords: Mitochondrial calcium uniporter, *C. elegans*, Neuron, Learning,

Memory

P-01-018

Nuclear hormone receptor NHR-49 in the body cavity neurons mediate pathogen avoidance in *C. elegans*

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Both fight and flight are important to survive in a harmful environment. In *C. elegans*, the nuclear hormone receptor NHR-49 is a functional homolog of mammalian PPAR and serve as an important regulator of fat metabolism. In addition to altered lipid composition, *nhr-49* mutants display a pleiotropy of defects, including shorter lifespan, impaired starvation response, and increased susceptibility to oxidative stress and pathogenic bacteria, hinting at the diverse roles that lipids play in the body. While trying to understand how NHR-49 controls immunity, we found that the mutant's susceptibility to the pathogen *Pseudomonas aeruginosa* (PA14) was largely due to defective avoidance response to the pathogenic lawn. Restoring NHR-49 in the neurons significantly improved the avoidance behavior, whereas intestinal rescue did not. Among the neurons, we found that restoring NHR-49 expression in cholinergic and glutamatergic neurons was sufficient for the increased avoidance. Genetic studies showed that NHR-49 acted downstream of the TGF β /DAF-7-mediated chemosensory detection of PA14, as well as the oxygen sensing pathway mediated through NPR-1. When we restored NHR-49 selectively in the body cavity AQR, PQR and URX neurons, pathogen avoidance significantly improved. Interestingly, genetic ablation of these three neurons also significantly improved pathogen avoidance, suggesting that NHR-49 is required for downregulating the activity of these neurons. We are currently trying to understand how NHR-49's role as a lipid regulator influences neuronal function and behavior.

Acknowledgement: *Pseudomonas aeruginosa* (PA14) is provided from prof. Seung-jae Lee's lab in KAIST.

Keywords: *Caenorhabditis elegans*, Nuclear hormone receptor, Neuron, Pathogen, Behavior

P-01-019

Rapamycin, an mTOR inhibitor suppresses orofacial neuropathic pain and p-mkk4/p-p38 MAPK-mediated microglial activation in TNC in trigeminal nerve injured mice

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Neuropathic pain caused by trigeminal nerve injury is a typical refractory orofacial chronic pain accompanied by the formation of hyperalgesia and allodynia. We previously demonstrated that rapamycin, an mTOR inhibitor suppressed orofacial formalin-induced nociception. However, it is unclear whether rapamycin can reduce trigeminal neuropathic pain and which mechanism is involved. In mice, infraorbital nerve was exposed, and partial nerve ligation (ION-PL) was performed using silk suture (8-0). At 14 days after surgery, neuropathic pain behaviors were examined on the whisker pad, and rapamycin (1 mg/kg) was intraperitoneally treated. The mechanical and cold sensitivity in left orofacial region was quantified using von-Frey filaments and acetone solution, respectively. The changes of mTOR and related proteins, p-mkk4 and p-p38 MAPK, GFAP and Iba-1 in TNC tissue were examined using western blot assay or immunohistochemistry. In addition, the cytokine assay was performed to verify the underlying mechanism for the anti-allodynic effect of rapamycin. Mice showed significant mechanical and cold allodynia at 2 weeks after ION-PL injury. Both mechanical and cold allodynia were significantly reduced 1 hour after rapamycin injection. In TNC tissue, ION-PL surgery or rapamycin treatment did not alter the p-mTOR,

p-S6 and p-4EBP1 expression, whereas rapamycin significantly decreased the increased expression of GFAP and Iba-1 in ION-PL mice. In addition, rapamycin suppressed the increase of p-p38 MAPK expression, which was related to decreased p-mkk4, but not p-mkk3/6 expression. In particular, the p-p38 MAPK positive cells were co-localized with the increased Iba-1, the microglia marker. Furthermore, rapamycin potently reduced cytokines and chemokines such as CXCL10, CXCL13, C5/C5a, IL-3, M-CSF and TNF-alpha in ION-PL mice. These findings demonstrated that rapamycin treatment reduced both facial mechanical and cold allodynia in trigeminal neuropathic mice, which was closely associated with the modulation of p-mkk4/p-p38 MAPK-mediated microglial activation in TNC. Moreover, the regulation of several inflammatory cytokines and chemokines were involved in these effects of rapamycin.

Keywords: Rapamycin, MTOR, Orofacial pain

P-01-020

Involvement of reactive oxygen species in cocaine addiction-like behavior in rats

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Repeated administration of cocaine induces neuroadaptation accompanied by enhanced behavioral activities in response to cocaine, a phenomenon known as behavioral sensitization. The increase in reactive oxygen species (ROS) in the brain caused by cocaine may contribute to cocaine-induced sensitization or toxicity. To determine whether an increase in reactive oxygen species (ROS) contributes to the development and maintenance of cocaine-induced behavioral sensitization, we examined the effects of ROS scavengers on the locomotor activity of rats following repeated cocaine administration using automated video tracking. To investigate the role of reactive oxygen species (ROS) in cocaine sensitization, the effect of a general ROS scavenger PBN (-phenyl-N-tert-butyl nitron) or superoxide specific scavenger TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidine-1 oxyl; 200 mg/kg, i.p.) was evaluated daily for 5 days and again for 19 days. Pre-treatment with either PBN or TEMPOL significantly and dose-dependently inhibited the locomotor activity induced by cocaine. After receiving PBN, the locomotor activity induced by intravenous cocaine was reversed. In addition, an injection of PBN into the dorsal striatum suppressed the increased locomotor activity induced by repeated cocaine administration. These effects of ROS scavengers were unaffected by systemic administration of diltiazem (a specific L-type calcium channel blocker), indicating that ROS may be generated via activation of dopamine receptors rather than an increase in intracellular Ca^{2+} via calcium channels. Re-exposure to cocaine induced locomotor activity, which was inhibited by a local injection of PBN into the dorsal striatum but not by NAC shall. In the striatum of cocaine-sensitized rats, immunofluorescence of 8-OHG was significantly higher than in saline-treated rats. The double labeling of cells expressing 8-OHG with NeuN indicates that striatal neurons produced ROS. After systemic injection of PBN (100 mg/kg, i.p.), the increased neuronal firing rates induced by cocaine injection return to baseline levels. BH4 and Xanthine/Xanthine (ROS producer) increased striatal DA levels in acute brain slices. Apocynin and AEBSF inhibited cocaine-induced increases in striatal dopamine (DA) levels. It suggests that increased production of reactive oxygen species, particularly superoxide radicals, in the striatum is a key factor in the development and maintenance of cocaine-induced behavioral sensitization by regulating dopamine levels.

Keywords: Cocaine, Addiction-like behavior, Reactive oxygen species, ROS scavenger, Sensitization

P-01-021

Homeostatic plasticity of Purkinje cell excitability balances fear-related memory

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In the brain, two forms of plasticity, synaptic and intrinsic plasticity, are known to be neural substrates for learning and memory. The balance between synaptic transmission and intrinsic excitability is important because abnormality in homeostatic plasticity causes severe diseases such as epilepsy, chronic pain, and Alzheimer's disease. Here, using patch-clamp recordings, we investigated homeostatic plasticity in the cerebellum related to fear memory. We found that the intrinsic excitability of Purkinje cells (PCs) decreases after auditory fear conditioning, which is known to potentiate the synapse between parallel fiber (PF) and PC. Regardless of whether the fear memory is formed or not, the activity of PCs evoked by PF stimulations was not significantly different. Depression of excitability was mediated by SK2 channel upregulation, which leads to prolonged afterhyperpolarization potential. Consistently, pharmacological blockade of SK2 channel after fear conditioning induced abnormal encoding of fear, implicating post-traumatic stress disorder. Furthermore, temporally limited optogenetic manipulation revealed that abnormal activity of PCs during the early consolidation period impairs normal freezing behavior. These results show that information related to fear memory is enhanced by synaptic potentiation, but counter-balanced by decreased intrinsic excitability to maintain the stable PC activity and sustain the fear memory in a normal range.

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Keywords: Homeostatic plasticity, Fear memory, Purkinje cell, SK2 channel, Intrinsic plasticity

P-01-022

Effect of monosodium urate on the terminal of substantia gelatinosa neurons of the trigeminal subnucleus caudalis in juvenile mice

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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. 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. 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 pipette solution, MSU was applied alone or with γ -aminobutyric acid (GABA), muscimol, or α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA). 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 a concentration-dependent manner under the high chloride pipette solution. Furthermore, the postsynaptic effect of MSU with GABA or muscimol, a GABAA agonist was decreased the amplitude on the SG neurons. But, the postsynaptic effect of MSU with AMPA was increased the amplitude on the SG neurons. These results suggest that MSU acts on the terminal of SG neurons of the Vc

by enhancing the AMPA-induced modulating activity for orofacial pain. Taken together, MSU contributes toward at least a part of orofacial nociceptive modulation and might be a promising target to develop therapeutic agents in orofacial pain treatment.

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Keywords: Orofacial pain, Whole-cell patch-clamp, GABA, Muscimol, AMPA

P-01-023

Effect of alpha-lipoic acid on substantia gelatinosa neurons of the trigeminal subnucleus caudalis in mice

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Alpha-lipoic acid (ALA) is a powerful antioxidant that occurs naturally in the body and metal ion chelator for metals. Numerous animal studies have confirmed that ALA has analgesic effects for pain, including burning mouth syndrome. However, little is known about the mechanism by which ALA exerts its analgesic effect at the central level. The substantia gelatinosa (SG) of the trigeminal subnucleus caudalis (Vc) play an important role in the processing and transmission of orofacial nociceptive information. This study aimed to investigate the direct effect of ALA and the action mechanisms on the SG neurons of the Vc by using patch-clamp technique.

Under the condition of high chloride pipette solution, bath application of ALA (300 μ M) repeatedly induced inward currents without desensitization in most neurons tested. ALA-induced inward currents were maintained in the presence of tetrodotoxin (0.5 μ M). In addition, ALA-induced inward currents were not affected by CNQX and DL-AP5 (ionotropic glutamate receptor antagonists), picrotoxin (GABAA receptor antagonist) and strychnine (glycine receptor antagonist). Therefore, in order to understand the mechanism of the analgesic effect of ALA, it is necessary to further study which receptors ALA is related to in SG neurons.

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Keywords: Patch-clamp, GABA, Glycine, Glutamate, Burning mouth syndrome

P-01-024

The inhibition of neuronal peroxisome proliferator-activated receptor- γ attenuates motor function improvement after spinal cord injury in rats

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Traumatic spinal cord injury (SCI) causes temporal deprivation of oxygen and glucose in the injured area. This leads to neuronal cell death as a result of secondary damage, which is one of the factors limiting motor function recovery after SCI. Peroxisome proliferator-activated receptor γ (PPAR γ) regulates critical cell survival mechanisms such as hypoxia, oxidative stress, inflammation, and energy homeostasis in various tissues. But the role of spinal PPAR γ after SCI is not well understood.

Under isoflurane inhalation, a 10g rod was freely dropped onto the exposed

spinal cord after the T10 laminectomy using a New York University impactor in the male Sprague-Dawley rats. We analyzed the cellular localization of spinal PPAR γ , locomotor function, and mRNA levels of various genes, including NF κ B-targeted pro-inflammatory mediators, after intrathecal administration of PPAR γ antagonists, agonists, or vehicles in SCI rats.

In both sham and SCI rats, spinal PPAR γ was located in neurons, not in microglia and astrocytes. The inhibition of PPAR γ induced motor neuron swelling with the mRNA levels of pro-inflammatory mediators and suppressed the recovery of locomotor function with a myelin-related gene expression in SCI rats. However, a PPAR γ agonist showed no beneficial effect on locomotor performance in SCI rats, despite a further increase in the protein expression of PPAR γ .

In conclusion, inhibition of PPAR γ may have negative influences on motor neuron survival and motor recovery. Nonetheless, exogenous PPAR γ activation does not appear to effectively help with functional improvement after SCI.

P-02-001

N-AS-triggered SPMs are direct regulators of microglia in a mouse of Alzheimer's disease

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Background: Sphingosine kinase1 (SphK1) is an acetyl-CoA dependent acetyltransferase which acts on cyclooxygenase2 (COX2) in neurons in a model of Alzheimer's disease (AD). However, the mechanism underlying this activity was unexplored.

Methods: For confirmation of COX2 acetylation-derived SPMs secretion by SphK1, we identified several SPMs, including 15-R-LxA4, 15-S-LxA4, 17-R-RvD1, 17-S-RvD1, 18-R-RvE1 and PD1, using systematic LC-MS/MS.

Results: Here we show that N-acetyl sphingosine (N-AS) is first generated by acetyl-CoA and sphingosine through SphK1. N-AS then acetylates serine 565 (S565) of COX2, and the N-AS-acetylated COX2 induces the production of specialized pro-resolving mediators (SPMs). In a mouse model of AD, microglia show a reduction in N-AS generation, leading to decreased acetyl-S565 COX2 and SPM production. Treatment with N-AS increases acetylated COX2 and N-AS-triggered SPMs in microglia of AD mice, leading to resolution of neuroinflammation, an increase in microglial phagocytosis, and improved memory. In addition, amyloid beta treated human microglia also showed reduction of N-AS generation, and N-AS treatment of these human cells improved SPM production and phagocytosis capacity as well.

Conclusions: Overall, these results reveal a biosynthetic mechanism and function of N-AS, which leads to S565 acetylation of COX2 and production of SPMs. They also reveal the relation of N-AS with microglial regulation in AD pathogenesis, and suggest a potential therapy for neuroinflammatory diseases, such as AD, using N-AS or related derivatives that could be evaluated in the future.

Keywords: Acetyl-CoA, N-acetyl sphingosine, COX2 acetylation, Production of SPMs, Neuroinflammatory resolution, Microglial phagocytosis

P-02-002

Discovery of a novel dual-action small molecule that improves multiple Alzheimer's disease pathologies

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Background: The importance of acid sphingomyelinase (ASM) activation has been recognized as a contributor to multiple Alzheimer's disease (AD)

pathologies, leading to the concept of using ASM inhibitors for AD treatment.

Methods: Chemical compounds (1,273) were tested in AD fibroblasts with abundance ASM activity. The compounds backbone with 30% inhibition was identified and optimization was performed based on lipophilicity. Further qualification was performed through biochemical and cellular assays, drug ability, and in vivo efficacy.

Results: We found KARI 201 with selectivity ASM inhibition effects, excellent pharmacokinetic properties, and especially brain distribution. Unexpectedly, another role of KARI 201 was revealed as a ghrelin receptor agonist, which has novel therapeutic potential for AD. This dual role of KARI 201 in neurons efficiently rescued multiple pathologies in AD mice, leading to memory function improvement.

Discussion: Our data highlights the potential clinical application of KARI 201 as innovative and multi-faceted drug for AD treatment.

Keywords: Alzheimer's disease, ASM direct inhibitor, GHSR1 alpha agonist, Memory improvement, Small compound

P-02-003

Chronic obstructive sleep apnea induces miRNA expression profiles associated with Alzheimer's disease in male rat

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Obstructive sleep apnea (OSA) is a common disorder which is associated with increased cerebrovascular disease and neurocognitive impairment. Although reports on cardio-vascular and cerebrovascular morbidity have been conducted, there are few research on the mechanism related to cognitive decline and dementia especially, Alzheimer's disease (AD). Recent study reinforces OSA-AD link with amyloid deposition in OSA brain and APOE genotype prevalence in OSA. This study is designed to find a link between OSA and neurodegenerative with successful animal model setting. A total of 16 rats were used, divided into control and OSA group. For OSA, 0.3 % cross-linked hyaluronic acid were injected twice every 12 weeks into the base of the tongue to create a OSA model. After 12 and 24 weeks, chronic OSA was identified with full channel polysomnography (PSG). The Morris water maze (MWM) test was conducted in control and OSA groups at 22 weeks, and pathological findings were subsequently confirmed 24 weeks after OSA induction. In addition, we studied the epigenetic changes with miRNA to identify the biomarkers for prediction of dementia in OSA. In MWM test, the speed of finding the platform was lower than that of the control group (47.0±13.9 sec; OSA group/ 12.4±6.1 sec; control, p<0.01). In brain histopathologic changes, disorganized cortex layers in OSA group were prominent compared with control cortex. Hippocampal cortex in OSA also showed a disorganized CA1 region with degenerative neurons and fibrillary changes, compared with control. The miRNA analysis identified an up-regulation in MiR-132-5p/137-3p/137-5p/501-3p, also known as AD, and a down-regulation of MiR-182/183-3p/183-5p/200a-3p/200b-3p/21-5p. OSA rat model with tongue hypertrophy showed neurodegenerative changes in brain and the epigenetic changes of mRNA/miRNA which have been known as AD related genes.

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Keywords: Obstructive sleep apnea, Alzheimer's disease, Neurodegeneration, Cognitive impairment

P-02-004

Short-term administration of Poria cocos extracts enhances sleep quality in rodent models with sleep disturbance

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Sleep disorders may have various causes and can incur mental and/or physical symptoms, and affect an individual's quality of life. In this study, we confirm that the Poria cocos extract (PCET) can improve sleep quality and structure by promoting inhibitory neurotransmission via the γ-aminobutyric acid (GABA) type A (GABAA) receptors based on the mechanisms revealed in the experiment with superior cervical ganglion neurons. Pento-barbital-induced sleep tests were conducted in order to determine whether the PCET extract improves the sleep quality and structure in normal ICR mice. Sleep latency and duration were checked with the righting reflex. To simulate the state of awakening as well as a normal sleep state, caffeine was administered orally before the PCET diet. After oral gavage of PCET, sleep latency was decreased, and total sleep duration was increased in normal and caffeine-induced sleep disturbance state. In the ACTH-induced sleep disturbed models, administration of PCET significantly reduced the sleep latency and increased the non-REM sleep duration, which was analyzed in real-time EEG by im-planting wireless electrodes in SD rats. PCET was found to improve the sleep quality under a normal sleep state through the GABAA receptor; it also promoted and improved the sleep quality and sleep structure in both the arousal activation state and stress-based sleep disturbance.

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Keywords: Poria cocos, Insomnia, Sleep disturbance, GABA, Caffeine

P-02-005

Green tea epigallocatechin-3-gallate (EGCG) improves hippocampal neurogenesis and memory performance impaired by X-irradiation in mice

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Neurogenesis is crucial to life-long generation of neural cells. It is affected by various factors such as genetic composition, growth factors and the environment. In mammals, increased regeneration of new granule neurons is associated with development of memory abilities, while decreased hippocampal neurogenesis results in impaired memory function in several neurological disorders. X-irradiation is commonly used to ablate tumor cells. It is also used as an immunosuppressive regimen in preparation for bone marrow transplantation. However, it affects adult neurogenesis in the brain which can result in neurodegenerative disorders. (-)-Epigallocatechin gallate (EGCG) is a major catechin found in green tea that can potentially have therapeutic benefit for persons with radiation-induced nonspecific damage to normal cells. However, the precise molecular mechanism underlying EGCG's beneficial effects on adult neurogenesis and memory performances in the hippocampal dentate gyrus (DG) after X-irradiation has not been well elucidated yet. Here, we report that damages caused by X-irradiation

such as decreased hippocampal neurogenesis and increased number of microglia activation were attenuated by EGCG in adult mouse hippocampal DG. These advantageous effects of EGCG occurred through inhibition of neuroinflammation by down-regulating X-irradiation-activated toll-like receptor 4 (TLR4)/NF κ B pathway followed by suppression of proinflammatory cytokines TNF- α , IL-1 β and IL-6. These results suggest that EGCG can restore adult hippocampal neurogenesis including memory system in the DG impaired by X-irradiation by compromising the TLR4/NF κ B signaling pathway in microglia. Thus, the manipulating immune response might be a potential therapeutic strategy to repair nonspecific X-irradiated damages in the central nervous system.

Keywords: Neural stem cell, Microglia, EGCG, Neurogenesis, X-irradiation

P-02-006

Reactive microglia and mitochondrial unfolded protein response following ventriculomegaly and behavior defects in 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- κ B signaling and apoptotic cell death appeared at the 5-day. Taken together, our findings demonstrate that activation of microglia and UPRmt occurs after hydrocephalic ventricular expansion and behavioral abnormalities which could be lead to apoptotic neuronal cell death, providing a new perspective on the pathogenic mechanism of hydrocephalus.

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Keywords: Hydrocephalus, Microglia, Neuroinflammation, UPRmt

P-02-007

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.

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Keywords: Vascular cognitive impairment, Vagus nerve stimulation, Cerebrospinal fluid, Glymphatic system

P-02-008

Bicarbonate permeability of synaptic GABAAR mediates neuronal excitation

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Ion permeation through anion channels plays essential roles in our body. However, how the anion channels preserve ion selectivity and how the anion selectivity is regulated are still poorly known.

Ionotropic GABAARs are anion selective channels activated by inhibitory neurotransmitter GABA. The $\alpha 1$, $\beta 3$, and $\gamma 2L$ subunits of hGABAARs were expressed in HEK 293T cells, and whole-cell recordings were performed. PHCO₃/PCI of hGABAARs was approximately 0.15 in response to 1 μ M GABA. Notably, a high concentration of GABA stimulation (1 mM) led to a biphasic increase (a peak followed by a small sustained elevation) in PHCO₃/PCI. We studied the PHCO₃/PCI increase in the GABAAR was associated with increased ϵ . Because of an extremely small permeability to F⁻, the increase in ϵ was more evident when the permeability value of F⁻ was excluded. We performed whole-cell recordings on pyramidal neurons in adult rodent sensorimotor cortical slices with moderately elevated [Cl⁻]_i (22 mM) and strong GABA stimulation (10 mM). The 10 mM GABA puff evoked an average depolarization of 21.1 mV in the HCO₃⁻-containing solutions and 12.4 mV in the HEPES-buffered solutions (5.5 mV in the solutions equilibrated with 100% O₂), suggesting that the PHCO₃/PCI of GABAARs reached 0.7 at this state. Surprisingly, the 21.1-mV depolarization in the HCO₃⁻-containing solutions evoked action potentials in five of the seven experiments. Conversely, action potentials were not observed in any of the ten experiments with HEPES-buffered solutions, indicating that the HCO₃⁻-efflux is critical to the GABAARs-mediated excitation in pyramidal neurons.

In conclusion, we provide evidence that ion selectivity of anion channels are determined by electric field strength and pore size. Specifically, agents that target HCO₃⁻ permeation in GABAARs may help treat neuronal diseases such as epilepsy.

Keywords: GABA, Bicarbonate, Epilepsy

P-02-009

Transcriptional alterations of TRPC1/C5 channel in Huntington knock-in striatal cells accelerate Ca²⁺-dependent cytotoxicity by Diamide-induced oxidative stressHana Lee¹, Insuk So², Chansik Hong¹¹Department of Physiology Chosun University College of Medicine, Gwangju, South Korea, ²Department of Physiology Seoul National University College of Medicine, Seoul, South Korea

Most transient receptor potential (TRP) channels are nonselective channels with Ca²⁺ permeability, which are emerging as potential targets for neurological disorders. Huntington's disease (HD) is a fatal hereditary and progressive neuronal degenerative disorder caused by a polyglutamine (polyQ) expansion in the protein huntingtin (Htt). Many studies have reported that polyQ aggregates increase the Htt-DNA interactions, which can lead to transcriptional dysregulation as well as translational disruptions. Although disrupted Ca²⁺ signaling is highly relevant in the pathogenesis of HD, the transcriptional defects in expression of proteins involved in the maintenance of intracellular calcium homeostasis are still not well understood. We previously identified altered expression levels and activities of redox-sensitive TRPC (classical type of TRP) channels in STHdhQ111/Q111 (Q111) HD model cells derived from mutant htt knock-in mice compared to wild-type STHdhQ7/Q7 (Q7) cells. To investigate the endogeneity inherent of intracellular Ca²⁺ dysregulation to oxidative stress between the onset and progression of HD, we performed RNA sequencing (RNA-Seq) to identify the differentially expressed genes (DEGs). We found that some genes in Q111 were susceptible to Ca²⁺-mediated toxicity induced by oxidative stress, which was consistent with public data on striatum tissues from HD patients. To clarify spatiotemporal Ca²⁺ dynamics by oxidative stress, we examined changes in diamide-induced Ca²⁺ mobilization from cellular influx to ER refilling in Q111 HD cells. Finally, pharmacological inhibition of TRPC5 attenuated diamide-induced apoptosis by rescuing the elevated Ca²⁺ and signaling. Taken together, our results suggest that aberrant TRPC1/C5 gene expression triggered by the inherited mutant Htt, accelerates and worsens HD disease progression. Considering that TRPC1/C5 channels are key regulators in HD pathogenesis, we propose timely clinical therapeutic intervention may help in ameliorating HD pathology.

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Keywords: Calcium, Cell death, HD, Oxidative stress, TRPC

P-02-010

Hyperactive ERK signaling in astrocytes impairs hippocampal learning and memoryMinkyung Kang^{1,2}, Jeongho Han³, Jihye Choi⁴, Hyun-Hee Ryu¹, Sunyong Kim^{1,2}, Kyoung-Doo Hwang^{1,2}, Jaegwon Lee^{1,2}, Pojeong Park⁵, Ja Eun Choi⁵, DaeHee Han⁵, Sang Jeong Kim^{1,2,7}, Bong-Kiun Kaang⁵, Benjamin G. Neel⁶, Chul Hoon Kim⁴, Hyungju Park³, Yong-Seok Lee^{1,2,7}

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Germline mutations in RAS signaling pathway are associated with various developmental disorders such as Noonan syndrome, neurofibromatosis, Cardio-facio-cutaneous syndrome (CFCs) and so on, collectively called RASopathy. Mutations in BRAF gene account for majority of CFCs. Most

of CFCs patients display severe degrees of cognitive impairments such as intellectual disability, but the neurobiological basis of their cognitive abnormalities remains largely unknown. Here, we investigated how the aberrant BRAF signaling affects cognitive functions using conditional knock-in mice harboring CFCs-associated BRAF mutations. Expressing the mutant BRAF under control of Nestin-CRE, resulted in severe hippocampal memory deficits in mice. Intriguingly, we found that the mutant BRAF increased the number of reactive-like astrocytes in multiple experimental systems including mutant mice, cultured mouse astrocyte, and human cortical organoid, suggesting that the mutant BRAF and subsequently activated ERK signaling renders astrocytes being in reactive-like status. Importantly, astrocyte-specific expression of the mutant BRAF in adult was sufficient to induce cellular and behavioral deficits in mice. Our study demonstrates that the reactive-like astrogliosis may underlies the severe cognitive deficits in CFCs.

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Keywords: Hippocampus, RAS-ERK signaling, Astrocyte, Neurodevelopmental disorder

P-02-011

ASD-like phenotypes in a mouse model of Noonan syndromeSoobin Kim^{1,2}, Sohyeon Park³, Gaeun Park^{1,2}, Minkyung Kang^{1,2}, Jae Jin Shin^{1,2}, Sang Jeong Kim^{1,2}, Moo Kyun Park⁴, Yong-Seok Lee^{1,2}

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Disruptions in sensory processing are common in autism spectrum disorder (ASD). Sensory processing impairments may lead to sensory hypo- and hyper-responsivity, which severely disrupts ASD patients' daily life. Among sensory problems, ASD is often associated with deficits in sensory habituation including habituation to acoustic stimuli. Noonan syndrome (NS) is a developmental disorder affecting sensory and cognitive functions. Importantly, recent studies showed that attention deficit hyperactivity disorder and social deficits are highly associated with NS. Previously, we have shown that a mouse model of NS, Ptpn11D61G/+, showed deficits in learning and memory. In this study, we found that the NS mice also show ASD-like behaviors, not only social behavior deficits but also abnormal sensory processing. NS mice showed abnormally increased hearing thresholds of auditory brainstem response (ABR) and decreased its amplitude of peripheral brainstem responses. Interestingly, NS male mice, not female, shows significantly impaired acoustic startle habituation and pre-pulse inhibition. Although the underlying mechanism for these sex specific-phenotypes remains to be investigated, our study demonstrates that the NS mutant mice show ASD-like behavioral phenotypes.

Acknowledgement: NRF-2019R1A2C1084232.

Keywords: Autism spectrum disorder, Sensory processing, Noonan syndrome, RASopathy

P-02-012

ASD-like phenotypes in a mouse model of Noonan syndrome

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Disruptions in sensory processing are common in autism spectrum disorder (ASD). Sensory processing impairments may lead to sensory hypo- and hyper-responsivity, which severely disrupts ASD patients' daily life. Among sensory problems, ASD is often associated with deficits in sensory habituation including habituation to acoustic stimuli. Noonan syndrome (NS) is a developmental disorder affecting sensory and cognitive functions. Importantly, recent studies showed that attention deficit hyperactivity disorder and social deficits are highly associated with NS. Previously, we have shown that a mouse model of NS, Ptpn11D61G/+, showed deficits in learning and memory. In this study, we found that the NS mice also show ASD-like behaviors, not only social behavior deficits but also abnormal sensory processing. NS mice showed abnormally increased hearing thresholds of auditory brainstem response (ABR) and decreased its amplitude of peripheral brainstem responses. Interestingly, NS male mice, not female, shows significantly impaired acoustic startle habituation and pre-pulse inhibition. Although the underlying mechanism for these sex specific-phenotypes remains to be investigated, our study demonstrates that the NS mutant mice show ASD-like behavioral phenotypes.

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Keywords: Autism spectrum disorder, Sensory processing, Noonan syndrome, RASopathy

P-02-013

Neuroinflammation and microglial NOD2/RIPK2 signaling in Parkinson's disease

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Neuroinflammation has been implicated as a major pathophysiological process of Parkinson's disease (PD). Among various neuroinflammatory triggers, α -synuclein (α -syn) aggregates have been considered as a predominant pathological trigger for microglial activation in the brain. However, the cellular mechanisms underlying microglia-mediated neuroinflammatory events following stimulation with α -syn aggregates have not yet been fully established. To gain a comprehensive understanding of the cellular mechanisms of α -syn aggregates induced microglial activation, we conducted RNAseq analysis using α -synuclein preformed fibrils (α -syn PFF) activated microglia. The analysis revealed that nucleotide-binding oligomerization domain-containing protein 2 (NOD2), pattern recognition receptor (PRR) of the NOD-like receptor (NLR) family and Receptor Interacting Serine/Threonine Kinase 2 (RIPK2), a kinase that functions as downstream of NOD2 and a potent activator of NF- κ B and inducer of pro-inflammatory cytokines to immune response are top-ranked. In our preliminary studies, we found that NOD2 and RIPK2 are strongly expressed in microglia in the substantia nigra (SN) of PD cases. Moreover, we found that α -syn aggregates activate NOD2/RIPK2 mediated inflammatory responses in microglia to exacerbates neuronal death through a series of signaling events including M1 microglial activation and neurotoxic reactive astrocytes conversion (A1 astrocytes) by secreting IL-1 α , TNF α and C1q (reported as A1 astrocyte inducer). Based on this data, we propose the role of NOD2/RIPK2 signaling as a key mediator

of inflammatory response in PD. The study will provide new insights into neuroinflammatory mechanisms and leads to development of novel therapeutic strategies for PD.

Keywords: Neuroinflammation, Parkinson's disease, Neurotoxic reactive astrocyte

P-02-014

Downregulation of TREK channels alleviates cognitive impairment in a mouse model of A β ₁₋₄₂-induced Alzheimer's disease

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Alzheimer's disease (AD) is a neurodegenerative disease with cognitive decline and memory loss. In AD, increased oxidative stress and inflammation due to excessive accumulation of amyloid beta protein, composed of 40 to 42 amino acids (A β ₁₋₄₂), are associated with neuronal and glial cell death. Dysregulation of TREK (TREK-1 and TREK-2) K_{2P} channels has been associated with epilepsy and depression. Depression is common in AD patients. Although many studies have demonstrated the role of the TREK channels in depression, a direct association between AD and the TREK channel has not yet been reported. This study was performed to identify the role of TREK channels in an A β ₁₋₄₂-injected mouse model. The AD biomarkers changed in response to A β ₁₋₄₂ were compared between wild-type (WT) and TREK deficient mice (TREK^{-/-}). Experimental groups were divided into WT, A β ₁₋₄₂-injected WT (WT-A β ₁₋₄₂), TREK^{-/-}, and A β ₁₋₄₂-injected TREK^{-/-} (TREK^{-/-}-A β ₁₋₄₂). In the WT-A β ₁₋₄₂, the degree of short-term memory and motor ability was decreased compared with the WT, as judged by Y-maze, forced swim, and Morris water maze tests. In addition, in the hippocampal region of the WT-A β ₁₋₄₂, the expression of BACE1 and tau protein and activation of microglia and astrocytes were significantly increased compared to that of the WT. TREK protein expression levels were highly enriched in the WT-A β ₁₋₄₂. In TREK^{-/-}-A β ₁₋₄₂, the alternation rate and mobility time were similar to the TREK^{-/-}. TREK^{-/-} showed increased alternation rate and mobility time compared with the WT. In the TREK^{-/-}-A β ₁₋₄₂, BACE1 and tau protein expression and microglia and astrocytes activation were insignificantly increased compared to TREK^{-/-}. Protein expression of MAP2 and BDNF decreased in WT-A β ₁₋₄₂, whereas their expression level was not significantly reduced in the TREK^{-/-}-A β ₁₋₄₂. Apoptosis signals, inflammation, astrocyte reactivation, ROS, and calcium concentrations were high in WT-A β ₁₋₄₂, while the markers were not high in TREK^{-/-}-A β ₁₋₄₂. Hippocampal primary cultured cells transfected with TREK-1 and TREK-2 siRNA showed lower A β ₁₋₄₂-induced apoptosis than scrambled siRNA-transfected cells. TREK channel blockers (paroxetine, fluoxetine) also showed similar effects to TREK siRNA. These results showed that the downregulation of the TREK channel inhibited A β ₁₋₄₂-induced AD behavior and biomarkers. Maintaining a steady-state expression level of the TREK channel contributes to suppressing the progression of cognitive impairment induced by A β ₁₋₄₂.

Keywords: Alzheimer's disease, Hippocampus, TREK channels

P-02-015

Peripheral Substance P induces hippocampal memory deficits**Sun Yong Kim**^{1,2}, Kyeong-No Yoon^{2,5}, Dong Hun Lee^{2,4,5}, Yong Seok Lee^{1,2,4}¹Department of Physiology Seoul National University College of Medicine, Seoul, Korea, ²Department of Biomedical Sciences Seoul National University College of Medicine, Seoul, Korea, ³Department of Biomedical Sciences, Neuroscience Research Institute Seoul National University College of Medicine, Seoul, Korea, ⁴Medical Research Center, Institute of Human-Environment Interface Biology Seoul National University, Seoul, Korea, ⁵Department of Dermatology Seoul National University College of Medicine, Seoul, Korea

Substance P is a neuropeptide that functions both in central nervous system and peripheral nervous system. Substance P neurons are well known to convey nociceptive information to the spinal cord and to regulate inflammatory responses in the peripheral tissues. However, the effects of peripheral increase of Substance P on hippocampal function such as spatial learning and memory are unknown. In this study, by subcutaneously injecting Substance P for 2 weeks and performing hippocampus-memory related behavior experiments, we found that peripheral increase of Substance P induces hippocampal memory deficits. Mice with 2 weeks of Substance P injection showed deficits in object place recognition and novel object recognition test compared to mice with saline injection. In addition, Substance P-injected mice showed tendency of impaired hippocampal LTP. Our data demonstrate that peripheral increase of Substance P affects hippocampus-dependent behaviors and plasticity.

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Keywords: Substance P, Synaptic plasticity, Hippocampus, Cognition, Learning and memory

P-03-001

Blockade of voltage-dependent K⁺ channels by olanzapine, atypical antipsychotic, in rabbit coronary arterial smooth muscle cells**Minji Kang**, Ryeon Heo, Seo-Yeong Mun, Wenwen Zhuang, Won Sun Park

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Olanzapine, an FDA-approved atypical antipsychotic, is widely used to treat schizophrenia and bipolar disorder. In this study, the inhibitory effect of olanzapine on voltage-dependent K⁺ (Kv) channels in rabbit coronary arterial smooth muscle cells was investigated. Olanzapine inhibited the Kv channels in a concentration-dependent manner with an IC₅₀ value of $7.76 \pm 1.80 \mu\text{M}$ and a Hill coefficient of 0.82 ± 0.09 . Although olanzapine did not change the steady-state activation curve, it shifted the inactivation curve to a more negative potential, suggesting that it inhibited Kv currents by affecting the voltage sensor of the Kv channel. Application of 1 or 2 Hz train pulses did not affect the olanzapine-induced inhibition of Kv channels, suggesting that its effect on Kv channels occurs in a use (state)-independent manner. Pretreatment with DPO-1 (Kv1.5 subtype inhibitor) reduced the olanzapine-induced inhibition of Kv currents. In addition, pretreatment with guangxitoxin (Kv2.1 subtype inhibitor) and linopiridine (Kv7 subtype inhibitor) partially decreased the degree of Kv current inhibition. Olanzapine induced membrane depolarization. From these results, we suggest that olanzapine inhibits the Kv channels in a concentration-dependent, but state-independent, manner by affecting the gating properties of Kv channels. The primary Kv channel target of olanzapine is the Kv1.5 subtype.

Keywords: Atypical antipsychotic, Olanzapine, Vascular smooth muscle cell, Voltage-dependent K⁺ channels

P-03-002

Inhibitory effects of the atypical antipsychotic, clozapine, on voltage-dependent K⁺ channels in rabbit coronary arterial smooth muscle cells**Minji Kang**, Seo-Yeong Mun, Ryeon Heo, Wenwen Zhuang, Won Sun Park

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To investigate the adverse effects of clozapine on cardiovascular ion channels, we examined the inhibitory effect of clozapine on voltage-dependent K⁺ (Kv) channels in rabbit coronary arterial smooth muscle cells. Clozapine-induced inhibition of Kv channels occurred in a concentration-dependent manner with an IC₅₀ value of $7.84 \pm 4.86 \mu\text{M}$ and a Hill coefficient of 0.47 ± 0.06 . Clozapine did not shift the steady-state activation or inactivation curves, suggesting that it inhibited Kv channels regardless of gating properties. Application of train pulses (1 and 2 Hz) progressively augmented the clozapine-induced inhibition of Kv channels in the presence of the drug. Furthermore, the recovery time constant from inactivation was increased in the presence of clozapine, suggesting that clozapine-induced inhibition of Kv channels is use (state)-dependent. Pretreatment of a Kv1.5 subtype inhibitor decreased the Kv current amplitudes, but additional application of clozapine did not further inhibit the Kv current. Pretreatment with Kv2.1 or Kv7 subtype inhibitors partially blocked the inhibitory effect of clozapine. Based on these results, we conclude that clozapine inhibits arterial Kv channels in a concentration- and use (state)-dependent manner. Kv1.5 is the major subtype involved in clozapine-induced inhibition of Kv channels, and Kv2.1 and Kv7 subtypes are partially involved.

Keywords: Clozapine, Coronary arterial smooth muscle cells, Voltage-dependent K⁺ channel, Use-dependent, Kv1.5

P-03-003

Plakophilin-2 deficiency augments Cx43 hemichannel-mediated ATP release and subsequent autocrine non-selective cation currents in HL-1 atrial myocytes under shear stress**Phuong Kim Luong**, Anh TV Vu, Qui A. Le, Sun-Hee Woo

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Cardiac hemodynamic disturbances are clinically associated with atrial arrhythmias and involve high shear stress onto atrial myocytes. However, the responses of atrial muscle to shear stress are poorly understood. We have previously reported that shear stress induces global Ca²⁺ waves in atrial myocytes that are sensitive to gap junction channel blockade. Mutations in plakophilin-2 (PKP2), the major component of desmosome, are the most common cause of familial arrhythmogenic right ventricular cardiomyopathy that also involves atrial arrhythmias. In this study, we tested whether PKP2 is involved in atrial shear stress response, mediated by connexin 43 (Cx43) gap junction protein, using PKP2-knockdown (KD) in HL-1 adult atrial cell line. The PKP2 proteins were detected in HL-1 cells as well as right and left atrial muscles from rats. Shear (~16 dyn/cm²)-induced ATP releases that were sensitive to gap 19 (Cx43 hemichannel inhibitory peptide), were significantly higher in PKP2-KD cells compared with wild-type (WT). Measurement of the efflux of calcein, a gap junction-permeable fluorescence dye, in single cells revealed that shear-triggered calcein efflux representing gap junction hemichannel function, was significantly augmented in PKP2-KD cells compared with WT. Shear-activated whole-cell cation currents were detected in HL-1 cells at negative potentials, which was eliminated by the treatment of gap 19 or ATP metabolizing enzyme (apyrase), suggesting roles of Cx43 hemichannels and autocrine ATP action on P2 purinoceptors. This shear-induced current was larger in PKP2-KD cells than WT cells. These data suggest that PKP2 deficiency may enhance Cx43 hemichannel opening and subsequent ATP release, thereby increasing membrane excitability in atrial cells under high shear stress.

Keywords: Plakophilin-2, Cx43 hemichannels, Atrial myocytes, ATP release, Cation currents

P-03-004

Asenapine, an atypical antipsychotic, blocks voltage-gated potassium channels in rabbit coronary artery smooth muscle cells

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We investigated the effect of asenapine, a commonly used atypical antipsychotic, on voltage-dependent K⁺ (Kv) channels in rabbit coronary artery smooth muscle cells. Asenapine inhibited the Kv current in a concentration-dependent manner, with an half-inhibitory concentration (IC₅₀) value of $8.59 \pm 2.25 \mu\text{M}$ and Hill coefficient of 0.64 ± 0.06 . Although asenapine did not affect the steady-state activation curve of Kv channels, it shifted the voltage dependence of the steady-state inactivation curve toward a more negative potential. Asenapine increased the recovery time constant of channel inactivation and produced use (state)-dependent inhibition of Kv channels at a stimulation frequency of 1 or 2 Hz. Pretreatment with the Kv1.5 subtype inhibitor DPO-1 reduced the Kv current; however, additional application of asenapine did not further inhibit the Kv current. Pretreatment with the Kv2.1 subtype inhibitor guangxitoxin and Kv7 inhibitor linopirdine also reduced the Kv current. However, additional application of asenapine further reduced the Kv current, similar to the application of asenapine alone. Asenapine induced membrane depolarization and vasoconstriction. Based on these results, we conclude that asenapine inhibits the Kv current in concentration- and use (state)-dependent manners by shifting the inactivation curve. The major target of asenapine is the Kv1.5 subtype channel.

Keywords: Asenapine, Voltage-dependent K⁺ channel, Use-dependent, Kv1.5

P-03-005

Calcium homeostasis modulator 2 (calhm2) is responsible for the slowly activating outwardly rectifying current in mouse B cells

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Previously, we found very slowly activating voltage-dependent current ($I_{V_{SAC}}$) in mouse B lymphocytes, which showed significant thermosensitivity, facilitation by alkaline pH, and inhibition by Gd³⁺ or ruthenium red (RuR) (Nam et al., 2006). Calcium homeostasis modulator 1 (calhm1) is a newly identified membrane protein forming nonselective voltage-dependent ion channel with slow kinetics, blocked by Gd³⁺ and RuR. The activation of calhm1 is facilitated by lowering extracellular Ca²⁺ concentration ($[\text{Ca}^{2+}]_{\text{ext}}$), physiological temperature, and alkaline pH (Jeon et al., 2021; Kwon et al., 2022). Based on the similarity with calhm1, we explored the molecular nature of $I_{V_{SAC}}$. RT-PCR analysis revealed the mRNAs of calhm2 and 6, while not calhm1, in the primary B cells and B cell lymphoma lines (WEHI-231 and BAL-17) of mice. Using whole-cell patch clamp, in addition to the thermosensitivity, facilitation of $I_{V_{SAC}}$ by lowering $[\text{Ca}^{2+}]_{\text{ext}}$ was confirmed. The overexpressed calhm2 in N2A cell also showed voltage-dependent slow activation, facilitation by temperature and alkaline pH. However, calhm6 overexpression did not show noticeable $I_{V_{SAC}}$ at 35 °C. Also, co-expression of calhm6 did not increase the amplitude of the calhm2 current. In BAL-17 cells, transfection with the anti-calhm2 siRNA significantly decreased the amplitude of $I_{V_{SAC}}$. Interestingly, no $I_{V_{SAC}}$ was recorded in the human primary B cells and cell lines despite the expression of the mRNA for CALHM2. Based

on the above results, we suggest that calhm2 is the molecular nature of $I_{V_{SAC}}$ in mouse B lymphocytes.

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Keywords: Calcium homeostasis modulator 2, CALHM2, B cells, Temperature sensitivity

P-03-006

Blockade of voltage-dependent K⁺ channels by the class Ic antiarrhythmic agent lorainide in coronary arterial smooth muscle cells

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Voltage-dependent K⁺ (Kv) channels play the role of returning the membrane potential to the resting state, thereby maintaining the vascular tone. Here, we used native smooth-muscle cells from rabbit coronary arteries to investigate the inhibitory effect of lorainide, a class Ic antiarrhythmic agent, on Kv channels. Lorainide inhibited Kv channels in a concentration-dependent manner with an IC₅₀ of $5.22 \pm 2.15 \mu\text{M}$ and a Hill coefficient of 0.92 ± 0.14 . Although application of lorainide did not change the activation curve, it shifted the inactivation curve toward a more negative potential, implying that lorainide inhibits Kv channels by changing the channels' voltage sensors. The recovery time constant from channel inactivation increased in the presence of lorainide. Furthermore, application of train steps (of 1 or 2 Hz) in the presence of lorainide progressively augmented the inhibition of Kv currents, implying that lorainide-induced inhibition of Kv channels is use (state)-dependent. Pretreatment with Kv1.5 or Kv2.1/2.2 inhibitors effectively reduced the amplitude of the Kv current but did not affect the inhibitory effect of lorainide. Based on these results, we conclude that lorainide inhibits vascular Kv channels in a concentration and use (state)-dependent manner by changing their inactivation gating properties. Considering the clinical efficacy of lorainide, and the pathophysiological significance of vascular Kv channels, our findings should be considered when prescribing lorainide to patients with arrhythmia and vascular disease

Keywords: Lorainide, Voltage-dependent K⁺ channels, Use dependency

P-03-007

Mass spectrometry-based identification of phosphorylation sites in Cav3.1 calcium channel and characterization of their roles by site-directed mutagenesis

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Cav3.1 T-type calcium channels play critical roles in neuronal low-threshold spikes, pacemaker activity, sleep control, and visceral pain. It has been

reported that protein kinases mediated phosphorylation regulates the activity and gating properties of T-type calcium channels. However, global identification of phosphorylation sites in Cav3.1 channel has been undetermined. In this study, we identified 30 phosphorylation sites (phosphosites) of rat Cav3.1 expressed in HEK-293 cells by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Site-directed mutagenesis of the identified phosphosites to Ala residues and functional studies of the phospho-silent mutants expressed in *Xenopus* oocytes revealed that the phospho-silent mutation of Ser18 residue in the N-terminus reduced its current amplitude, accelerated current kinetics, and negatively shifted steady-state inactivation curve. In addition, the phospho-silent mutation of Ser1924, Ser2001, Ser2163, Ser2166, or Ser2189 in the C-terminus greatly reduced their current amplitude without changing the voltage-dependent gating properties. In contrast, the phosphomimetic Asp mutations of them recovered or reversed the reduction effects of the phospho-silent mutations. Taken together, these findings suggest that the multiple phosphosites of Cav3.1 in the N- and C-termini play crucial roles in regulation of the activity and/or voltage-dependent gating properties of Cav3.1 channels.

Keywords: Cav3.1 T-type Calcium channel, Mass spectrometry, Phosphorylation, *Xenopus* oocytes, Voltage-clamping

P-03-008

Bi-directional 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 ($[\text{Ca}^{2+}]_e$). 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 non-additively 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, but not inhibition at acidic pHe, was use-dependent, suggesting that the sensitivity to pHe could be due to H^+ diffusion through the open-state CALHM1. At pHe 6.2, decreased $[\text{Ca}^{2+}]_e$ 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 $[\text{Ca}^{2+}]_e$ and acidic pHe. This study is the first to demonstrate the remarkable pH-sensitivity of CALHM1, which might contribute to the pH-dependent modulation of neuronal excitability or taste sensation.

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Keywords: CALHM1, Ion channel, Voltage-dependent activation, pH-sensitivity

P-03-009

Fucoxanthin suppresses NMDA and AMPA receptor-mediated excitation on substantia gelatinosa neurons of the trigeminal subnucleus caudalis in immature mice

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Fucoxanthin, a carotenoid compound, is a potential drug source extracted from brown seaweeds. This phytochemical compound has been known to exhibit various pharmaceutical properties, such as neuroprotective, anti-inflammatory, and anti-nociceptive effects. The substantia gelatinosa (SG) of the trigeminal subnucleus caudalis (Vc) has been recognized as a key site for regulating nociceptive transmission from the periphery. However, direct effect of fucoxanthin on the SG of the Vc and its mechanism of action have not been extensively clarified. Therefore, in this study, the whole-cell patch-clamp technique was applied to investigate effects of fucoxanthin on excitatory neurotransmitters such as glutamate receptor and its ionotropic receptors recorded from SG neurons of the Vc in mice. Under high chloride pipette solution, fucoxanthin suppressed glutamate receptor-activated ion current. Specifically, N-methyl-D-aspartic acid (NMDA)- and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-induced responses but not kainate receptor-mediated response were decreased by fucoxanthin in the voltage-clamp mode. Altogether, these results reveal that fucoxanthin could regulate SG neuronal activities in juvenile mice by partially inhibiting the excitatory signaling induced by NMDA and AMPA receptors. The suppressive effect of fucoxanthin on glutamate receptor-induced excitotoxicity on the SG of the Vc might be suggested as being the underlying orofacial-antinociceptive mechanism of fucoxanthin.

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Keywords: Marine product, Patch-clamp, Excitatory neurotransmitter, Glutamate receptor, Orofacial pain

P-03-010

G protein beta2 subunit regulates the activity and current kinetics of Cav3.3 T-type channel via its association with the Cav3.3 C-terminus

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Calcium entry through T-type calcium channels acts as signals triggering diverse cellular events. Previous studies reported that Cav3.2 channel activity was down-regulated by G protein beta2-gamma subunits via their interaction with Cav3.2 II-III loop, whereas Cav3.1 channel was not. Similarly, it was reported that activation of M1 Ach receptor down-regulated Cav3.3 channel in which beta-gamma subunits were involved. However, it remains elusive whether and how Cav3.3 channel is regulated by beta-gamma subunits. In this study, we investigated the mechanisms for the beta-gamma mediated regulation of Cav3.3 channel in HEK-293 cells in which Cav3.3 were reconstituted with or without beta-gamma dimers. Patch clamping recordings of HEK-293 cells showed that coexpression of G beta2-gamma2 reduced Cav3.3 currents in peak amplitude and accelerated current kinetics. These G beta2-gamma2 regulatory effects were mimicked by coexpression of G beta2, but lessened by coexpression of G beta1, G beta1-gamma2, or G gamma2, suggesting that G beta2 subunit is required for the regulation. We employed yeast-two-hybrid (Y2H) assays to look for Gbeta2 interaction sites, and identified that G beta2 subunit interacts with the C-terminus (CT)

of Cav3.3, but not with other cytoplasmic regions. Consistently, deletion of the Cav3.3 CT harboring the Gbeta2 interaction site abolished the Gbeta2-gamma2 regulation effects. Taken together, we report that G beta2 can down-regulate Cav3.3 channel activity and accelerate current kinetics via its association with the Cav3.3 CT.

Keywords: T-type calcium channel, G protein, Whole-cell patch clamp, Yeast-two-hybrid

P-03-011

Identification of a novel tricyclic antidepressant binding site within opioid receptor using molecular dynamics and functional assays for TRPC4

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Tricyclic antidepressants (TCAs) had been used as a first-line treatment for mood disorder, but rapidly vanished due to moderate-to-severe adverse effects. Instead, the TCAs showed some promising analgesic effects towards neuropathic diseases, and an outstanding therapeutic effect to patients who suffer from irritable bowel syndrome. However, the mechanism by which those atypical effect are manifested has been unclear. Among the proposed mechanisms were 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 commences the Gi signaling. Based on minimal-energy docking, we have also identified a putative TCA binding site within OR. Finally, we verified whether TCA-induced Gi-pathway could be modulated when the binding site was altered.

In cAMP ELISA to detect OR downstream products, treatment with DAMGO, μ OR agonist, or amitriptyline (AMI) decreased the concentration of intracellular cAMP compared to vehicle. In addition, the FRET efficiency between Gai and OR was also decreased when agonist and AMI were treated. Next, we predicted the binding site of TCA based on the previously revealed Cryo-EM structure of the activated OR. Within the binding site, an aspartate residue showed the most significant contribution towards the drug binding, and aspartate-to-arginine mutation of the residue severely decreased the FRET efficiency once robustly induced by AMI.

As an alternative way to monitor the Gi-signaling efficacy, we have utilized a well-known functional relationship between Gai protein and TRPC4. Namely, we measured TRPC4 current in HEK293 cells in which either WT or mutant ORs are co-expressed. All types of TCA elicited μ OR-induced TRPC4 current, as did the δ and κ ORs. In order to dissect whether the increased TRPC4 activity is indeed evoked from the TCA-OR-Gai pathway, we tried to deliberately block the functional link between OR and Gai by applying pertussis toxin, which interferes a native interaction between Gi-protein and GPCR, or over-expressing Gai2(G203T), a dominant-negative mutant. As expected, TCA-induced current of TRPC4 was not observed in the Asp-Arg mutant of OR.

In summary, we predicted the TCA binding site of OR, and performed a biochemical assay and electrophysiology to support the validity of the molecular dynamics-based binding site. Our findings demonstrate that OR could be proclaimed as a promising target among numerous binding partners of the TCA, and that TRPC4 activation could be expected as a significant downstream pathway.

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Keywords: Opioid receptor, Tricyclic antidepressants, TRPC4, Inhibitory GPCR, Receptor structure

P-03-012

Shear stress increases junctional Ca^{2+} sparks in atrial myocytes via NADPH oxidase 4-dependent mitochondrial ROS generation

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Cardiac myocytes are subjected to various mechanic stimulation, such as stretch and shear stress during the cardiac cycle. We have previously reported that shear stress increases reactive oxygen species (ROS) in the mitochondria via NADPH oxidase (Nox) 2, thereby increasing spark frequency in ventricular myocytes. In this study, we examined whether and how atrial myocytes show such shear responses and further investigated their remodeling under chronic pressure overload. We used mitochondrial superoxide indicator MitoSOX-Red-acetoxymethyl ester (AM) and intracellular Ca^{2+} dye fluo-4 AM with real-time two-dimensional confocal microscopy to measure mitochondrial ROS changes and Ca^{2+} sparks in isolated murine atrial myocytes under shear stress. In the atrial myocytes, mitochondrial ROS significantly increased by shear stress. Pretreatment of cells with the inhibitor of Nox, DPI (3 μM), or the mitochondrial ROS scavenger, mito-TEMPO (25 μM), inhibited the shear-induced ROS increase by about 95% and 80%, respectively. GKT831 (20 μM), the inhibitor of Nox4, suppressed the shear-induced ROS increase by about 85%. Furthermore, mitochondrial ROS increases in the presence of shear stress in Nox4 knock-out mouse atrial myocytes were only about 20% of those measured from wild-type mouse atrial cells. This shear-induced mitochondrial ROS increase was attenuated in atrial cells isolated from dilated left atria of rats due to chronic transverse aortic constriction. Consistently, in this failed atria, Nox4 expression was decreased. Shear-induced enhancement of Ca^{2+} spark frequency was observed in the junctional and non-junctional sites of atrial cells, which was significantly suppressed by mito-TEMPO. In Nox4 knock-out mouse atrial cells the stimulatory effects of shear stress on the spark occurrence were eliminated only in the peripheral sites, not in the central non-junctional sites. Our data suggest that shear stress may increase mitochondrial ROS via Nox4, thereby increasing Ca^{2+} spark occurrence in the peripheral junctions of atrial myocytes, and that this response was downregulated in the failed left atrial myocytes under chronic pressure overload.

Keywords: Shear stress, Atrial myocytes, Mitochondrial ROS, Ca^{2+} spark, Nox4, Chronic pressure overload

P-03-013

Inhibitory effect of benztropine, a muscarinic acetylcholine receptor inhibitor, on voltage-dependent K^{+} channels in 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 $6.11 \pm 0.80 \mu\text{M}$ 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

P-03-014

Hydrogen peroxide affects the post-synaptic GABA_A receptor-mediated neurotransmission on gonadotropin-releasing hormone neurons

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Gonadotropin-releasing hormone (GnRH)-secreting neurons receive synaptic inputs that include excitatory and inhibitory signals from various neurotransmitters and neuropeptides. GABAergic neurotransmission is regarded as the principal inhibitory neurotransmitter in the adult brain, even though it is excitatory in the early developmental stage. However, the role of GABAergic neurotransmission in adult GnRH neurons remains controversial. Reactive oxygen species (ROS) act as signaling molecules in several neuronal populations. However, its impact on GABAergic neurotransmission that regulates GnRH neuron physiology remains unknown. Thus, we investigated the effect of hydrogen peroxide (H₂O₂), a ROS source, on GABAergic signaling that regulates GnRH neuron physiology. We used whole-cell voltage clamp techniques to determine the effect of H₂O₂ on GABAergic signaling in GnRH neurons. H₂O₂ increased the frequency of spontaneous post-synaptic currents. The increased synaptic events were preserved in the presence of tetrodotoxin (a voltage-gated Na⁺ channel blocker) and 6-cyano-7-nitroquinoxaline-2,3-dione with DL-2-Amino-5-phosphonovaleric acid (glutamate receptor antagonists) suggesting an increase in miniature inhibitory post-synaptic currents. Furthermore, H₂O₂ enhanced synaptic events were entirely blocked by GABAA receptor antagonist bicuculline, indicating that increased synaptic events were GABAergic. Exogenous H₂O₂ increased GABAergic synaptic events on GnRH neurons via action potential independent presynaptic mechanism. Furthermore, H₂O₂ enhances the post-synaptic GABAA receptor's activity on GnRH neurons and regulates the GnRH neuronal excitability, potentially influencing gonadotropin's release from the hypothalamus.

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Keywords: Reactive oxygen species, GABAergic signaling, Patch-clamp, Miniature inhibitory post-synaptic currents, Hypothalamus

P-03-015

Trp434 and Trp435 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 7 subunits, TRPC4 and TRPC5 channels are well known to be potentiated by direct bindings of calcium

ion and phosphatidylinositol 4,5-bisphosphate. Both calcium and PIP2 were therefore also implicated in not only activation of the channel but also desensitization processes. The binding site for calcium ion was revealed but PIP2 binding site remains controversial. Previously, we found two tryptophan residues in S3 helix of TRPC4 channel as a candidate by which calcium sensing in S2-S3 linker is delivered to channel gating. Here, we suggest the WW site in TRPC5 channel conducts delivering of calcium sensing like in TRPC4, and also acts as a potential PIP2 binding site.

We made two single mutants (W434A, W435A) and a double mutant (WW/AA) from human TRPC5 channel. Wild type and mutant TRPC5 channels were expressed in HEK293 cells for macroscopic current recording in whole-cell or inside-out mode. All mutant channels showed typical doubly-rectifying I-V curves of TRPC5 channel when stimulated by non-physiological agonist, (-)-Englerin-A, in whole-cell mode. But under stimuli imitating physiological environment, W434A showed gain-of-function phenotype while W435A and the double mutant showed loss-of-function phenotypes. Interestingly, when W434A channel was co-expressed with human muscarinic acetylcholine receptor type 3 (mAChR3) and stimulated by carbachol, it didn't show desensitization which is characteristically observed in wild-type TRPC5 channel. To investigate direct interaction with PIP2, we delivered a water soluble form of PIP2 (diC8-PI(4,5)P2) to intracellular side of the patch in inside-out mode. As a result, W434A and W435A channels were not potentiated by intracellular PIP2.

Loss of desensitization in W434A mutant channel implies that this mutant has lost the ability of sensing PIP2 hydrolysis by PLC or the ability of phosphorylation by PKC. The latter possibility is ruled out because the C-terminus of the channels we used were truncated so they do not contain the phosphorylation site. Recently, PIP2-bound ion channel structures were revealed including TRPs. Many of them have PIP2 binding sites near S3, S4, and S5 helices. Our electrophysiological data also indicate that PIP2 binds near S3 helix in TRPC5 channel, and the WW site is a specific candidate for sensing PIP2 and calcium.

Keywords: TRPC channel, PIP2 binding, Ca²⁺ binding, Channel desensitization

P-03-016

Electrophysiological properties of TRPC1/4 heteromer determined by its pore residues

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Transient receptor potential canonical 1 (TRPC1) is widely expressed in various mammalian tissues and is involved in many physiological functions. It is known to heteromerize with TRPC4 to form a cationic channel, and it induces significant changes in channel characteristics. The huge differences in channel characteristics in the physiological environment indicates the importance of channel regulation in many tissues expressing both TRPC1 and TRPC4. In this study, we focused on the pore region (selectivity filter, pore helix, and S6 helix) of TRPC1 and TRPC4, compared their homology, and investigated how they contribute to the electrophysiological characteristics of the ion channel. We observed changes in G-V curve and I-V curve with mutations of the pore region and demonstrated that TRPC4 selectivity filter mutants lost sensitivity to G protein mediated activation. Moreover, lower gate mutants of TRPC4 exhibited diminished calcium permeability compared to wild type channels. Through chimeric channels where we substituted the pore region of TRPC1 with that of TRPC4, we were able to determine regions that are critical in producing the double-rectifying I-V curve of the homomeric TRPC4 channel.

Keywords: TRPC1, TRPC4

P-03-017

Activation of TRPV3 is required for keratinocyte differentiation and epidermal barrier formation

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Keratinocytes compose about 90% of the epidermis and their proper differentiation allows the skin to function as a barrier against water loss and environmental assaults. While the established calcium gradient and the elevated calcium level in the suprabasal layer of the epidermis is required for the initiation and progression of keratinocyte differentiation, the emerging role of calcium-permeant receptors and ion channels has been highlighted. Of particular interest is TRPV3, a member of the transient receptor potential (TRP) family, which shows a prominent expression in the skin. Here in this study, we investigated the role of TRPV3 in keratinocyte differentiation and epidermal barrier formation. In vitro differentiation of normal human epidermal keratinocytes (NHEKs) was induced via a Ca^{2+} -switch protocol and TRPV3 currents (ITRPV3) were recorded using the patch clamp technique in the whole-cell configuration. Along with the increased expression of TRPV3 in differentiated keratinocytes, the significant increase in ITRPV3 towards the terminal differentiation was observed. The immunoblot assay in NHEKs showed decreased markers by TRPV3 antagonists (ruthenium red) while transiently increased markers by agonists (2-APB). Upon application of 2-APB with another TRPV3 agonist (carvacrol), the skin barrier recovery test (TEWL) in mice showed enhanced recovery rate. These results reveal that TRPV3 is involved in the process of keratinocyte differentiation and its activation is required to maintain the integrity of the epidermal barrier.

Keywords: TRPV3, Keratinocyte differentiation, Epidermal barrier formation, Ion channels, Calcium

P-03-018

Molecular basis for PI(4,5)P2 modulation 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)P2) in the plasma membrane plays an important role in regulating the PAC channel activity. The depletion of PI(4,5)P2 level through Danio rerio voltage-sensitive phosphatase (Dr-VSP) activation suppressed both PAC1 and PAC2 channels. The suppression of PAC channels was seen in cells where the PI(4,5)P2, but not PI4P and PI(3,4,5)P3, were depleted. The suppression was also repeated by the activation of M1 muscarinic receptor (M1R), a Gq protein-coupled receptor, through the signaling pathway that hydrolyzes PI(4,5)P2 in both transiently and endogenously expression system. We also found that single point mutation of basic amino acids located at the interface between transmembrane domain 2 (TMD2) 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)P2-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)P2. Mutants on PI(4,5)P2-binding sites reduced acid-induced cell death due to suppressed PAC channel activity, indicating that regulation of PAC channel by PI(4,5)P2 affect cell death induced by acid treatment. Our study revealed that membrane PI(4,5)P2 is a key factor regulating the PAC channel gating.

Keywords: Proton-activated chloride channel, PAC channel, Phospholipid, PI(4,5)P2, Acid-induced cell death

P-03-019

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 $[\text{Na}^+]$ 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 $\text{NaV}1.2$, 1.3 , 1.6 sodium channels were significantly up-regulated in P12 rat hippocampus. Interestingly, expression of $\text{NaV}1.5$, 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: Neuronal development, Action potential, Voltage-gated Na^+ channels, Hippocampal neuron

P-03-020

Phosphate-mediated calcium regulation in podocyte integrity

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As the sentry of the kidney filtration barrier, podocyte conserves its actin cytoskeleton by intracellular Ca^{2+} signaling, whose disturbance by $\text{TrpC}5/6$ (transient receptor potential cation channel subfamily) results in slit diaphragm disruption and proteinuria. Recent evidence emphasizes the pivotal role of Orai1-mediated SOCE in preserving filter integrity related to podocyte injury and proteinuric diseases. Hyperphosphatemia is a primary complication of renal damage found in advanced chronic kidney disease (CKD). The impact of excessive inorganic phosphate (Pi) on SOCE-mediated Ca^{2+} signaling in podocyte actin dynamics and filtering function leading to proteinuria in CKD remains skeptical. Here we show that transient Pi-induced mitochondria-driven reactive oxygen species (ROS) production causes endoplasmic reticulum (ER) stress by triggering Ca^{2+} release, which further activates SOCE. Pi increased the surface abundance of Orai1 and TRPC6 via the Akt-dependent exocytosis of the channels. This cytosolic calcium dysregulation or ROS itself temporarily disintegrated podocyte actin cytoskeleton and reduced protein expression of synaptopodin, causing higher albumin permeability due to alteration in podocyte morphology or plasticity. Notably, inhibition of Orai1 by GSK7975A partly rescued this injury via actin dynamics and synaptopodin enhancement. Concurrently, short-term Pi treatment elevated the protein expression of several stress markers such as αKlotho , Gdf15, and Fgf21, which potentiate negative feedback to

hinder the fatal influence of ROS and coordinate cytosolic Ca^{2+} via SOCE or TRPC6. Nevertheless, our studies also reveal the impact of long-term Pi exposure to irreversible disruption of the podocyte actin cytoskeleton and cell death, likely resulting in loss of slit diaphragm integrity and proteinuria, subsequently. Overall, our data sheds light on both the positive and negative influences of Pi in podocytes, particularly in Ca^{2+} -regulated podocyte filter function.

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Keywords: SOCE, STIM1, Orai1, Trpc6, Ca^{2+} signaling, Actin cytoskeleton, Proteinuria, ROS

P-03-021

Protective effect of tomatidine against cardiac hypertrophy induced by isoproterenol in cellular system and electrophysiology

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Tomatidine is known to regulate mitochondrial functions, but it has not been well studied, including channel-related electrophysiology. The purpose of this study was to investigate the cardioprotective effect of Tomatidine (Toma) on the cardiac hypertrophy model induced by Isoproterenol (ISO). AC16 cardiomyocytes were used to test cell cytotoxicity using CCK-8 and ROS assay kits. Ventricular myocytes isolated from mouse hearts were used to measure calcium currents using the patch clamp technique. AC16 cells were treated with 200 mM ISO, and it was confirmed that no cytotoxicity occurred in each group when treated with 10 or 20 μM Toma. However, in the ROS assay, it was confirmed that Toma reduced the ROS induced by ISO in a concentration-dependent manner. In addition, the protein expression measurement experiment confirmed that cardiac hypertrophy occurred as the cardiac hypertrophy protein BNP increased, and BNP expression was decreased in the Toma group. The calcium currents of the ISO group were significantly different from those of other groups, and the Toma group showed a similar trend to that of the normal control group. These results indicate that Toma has a downregulating effect on ROS and modulates calcium channels in ISO-induced cardiac hypertrophy. Our results suggest that Tomatidine may be a prospective target for cardiac hypertrophy.

Keywords: Cardiac hypertrophy, Electrophysiology

P-03-022

α Klotho ameliorates podocyte injury and proteinuria in diabetic nephropathy via stabilizing podocyte Ca^{2+} channels

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α Klotho, an anti-aging protein produced in the kidney, is known to protect the kidney filter and renal diseases via regulating renal Ca^{2+} ion channels. Intracellular Ca^{2+} homeostasis in podocytes is vital for maintaining actin cytoskeleton structure, and excessive Ca^{2+} overload via TRPC5 or 6 channels has been implicated in proteinuria in diabetic nephropathy (DN). Recently, we reported that upregulation of Orai1-mediated store-operated Ca^{2+} entry in

a hyperinsulinemic early period of DN causes proteinuria, while TRPC5 and 6 channels were increased in the late period of DN leading to proteinuria. However, it is still unknown whether and how α Klotho regulates multiple podocyte Ca^{2+} channels to protect DN. Here we demonstrated that α Klotho ameliorates podocytes injury by stabilizing Orai1 and TRPC5/6 channels-mediated Ca^{2+} signaling to prevent DN. 11-week-old or 19-week-old type 2 diabetic db/db mice were used as the early or late period of DN model, respectively. The mice were administered with α Klotho recombinant peptide through i.p. injections. In both DN periods, α Klotho was reduced along with podocyte markers such as synaptopodin and nephrin, while Orai1, and TRPC5/6 were overexpressed in early and late periods in db/db mice, respectively. Administration of α Klotho protein ameliorated podocyte foot process disruption and proteinuria in db/db mice with decreased expression of channel proteins and the dissolution of synaptopodin. In vitro, α Klotho suppressed Orai1- and TRPC5/6-mediated Ca^{2+} entry in cultured murine podocytes via inhibiting growth factors and/or insulin signaling. Mechanistically, α Klotho acutely reduced cell surface abundance of Orai1 by suppressing phosphoinositide-3-kinase-dependent trafficking of the channel, however, TRPC5/6 channels were slowly decreased by inhibiting growth factor-driven SGK1 activation. All the total channel proteins were reduced in long-term treatment (~24 h) of α Klotho. Functionally, exacerbated actin remodeling by Orai1 and TRPC6 activation was ameliorated by α Klotho. Taken together, our results reveal an underlying mechanism by which α Klotho protects proteinuria and podocyte actin remodeling through stabilizing Ca^{2+} signaling mediated by Orai1 and TRPC5/6, and offer a new potential therapeutic strategy for the treatment of DN.

Acknowledgement: This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF-2017R1A5A2015369, 2022R1C1C2009853 & 2022R1A2C2011079).

Keywords: α Klotho, TRPC6, TRPC5, Orai1, Proteinuria, Podocyte, Diabetic nephropathy

P-03-023

NR2A-containing NMDARs detect increased ambient glutamate concentration in supraoptic nucleus of DOCA-salt hypertensive model rats

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The ionotropic NMDA receptors (NMDARs) modulate glutamatergic excitatory tone with their dual modality, the phasic and tonic currents in the brain. Here, we demonstrated that tonic NMDARs currents (INMDA) of magnocellular neurosecretory cells (MNCs) is an efficient biosensor detecting altered ambient glutamate level in the supraoptic nucleus (SON) of DOCA-salt hypertensive model rats. INMDA measured by nonselective NMDARs antagonist, AP5, at Vholding -70 mV in low concentration of ECF Mg^{2+} ($[\text{Mg}^{2+}]_o$) was transiently but significantly increased at 7 days of DOCA implant in DOCA-salt group. Total INMDA of non-depolarized SON MNCs was compatible to IPEAQX uncovered by PEAQX, a GluN2A-selective antagonist, thus, insensitive to GluN2B antagonist, ifenprodil or GluN2C/D antagonist, PPDA in both DOCA-H₂O and DOCA-salt rats. Increased ambient glutamate by exogenous glutamate or glutamate transporter (GLUT) antagonist, TBOA, demolished IPEAQX and INMDA difference between DOCA-H₂O and DOCA-salt group, suggesting that attenuated GLUT activity increased ambient glutamate concentration in DOCA-salt groups. In contrast, only ifenprodil but not PEAQX and PPDA uncovered INMDA at Vholding +40 mV under 1 mM $[\text{Mg}^{2+}]_o$ condition, while ifenprodil sensitive INMDA of depolarized SON MNCs was not different in DOCA-H₂O and DOCA-salt groups. Finally, GluN2A, GluN2B, and GluN2D protein expression was not different in the SON of the two groups. Taken together, NR2A-containing NMDARs mediating IPEAQX efficiently detected the increased ambient

glutamate concentration in the SON of DOCA-salt hypertensive model rats, which may contribute to ADH release with attenuated GLUT activity.

Keywords: NMDARs, NR2A, NR2B, Hypertension, SON

P-03-024

Roles of Zn^{2+} in Mg^{2+} -free-induced epileptiform activity in the CA3 region of rat hippocampal slices

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Hippocampal CA3 neurons receive excitatory input (i.e., mossy fibers) from dentate gyrus and inhibitory input from GABAergic interneurons. Zn^{2+} is co-released with glutamate from mossy fiber terminals spontaneously and with electrical stimulation. Zn^{2+} inhibits various ion channels including NMDA receptors and voltage-gated Ca^{2+} channels, and GABA transporter 4. In this study, we investigated roles of Zn^{2+} in interictal-like epileptiform activity in the CA3 regions of rat hippocampal slices without the entorhinal cortex using extracellular recordings. Exposure to Mg^{2+} -free artificial cerebrospinal fluid induced interictal-like epileptiform activity in the CA3 regions. The intracellular Zn^{2+} chelator TPEN (50 μ M) and the extracellular Zn^{2+} chelator CaEDTA (1 mM) both significantly increased the frequency of the interictal-like epileptiform activity, whereas $ZnCl_2$ (300 μ M) significantly decreased the epileptiform activity. The electrical stimulation of mossy fiber (0.05 Hz) decreased the epileptiform activity. Both TPEN and CaEDTA significantly blocked the electrical stimulation-induced inhibitory effects on the epileptiform activity. However, $ZnCl_2$ significantly potentiated the inhibitory effects of electrical stimulation on the epileptiform activity. The GABAA antagonist bicuculline (10 μ M) alone had no significant effects on the epileptiform activity. However, TPEN significantly increased the epileptiform activity in the presence of bicuculline. Bicuculline had no significant effects on the electrical stimulation-induced inhibitory effects on the epileptiform activity. However, TPEN significantly blocked the electrical stimulation-induced inhibitory effects on the epileptiform activity in the presence of bicuculline. All these results suggest that Zn^{2+} has an inhibitory effect on interictal-like epileptiform activity in the CA3 region of the rat hippocampus, presumably via glutamatergic or GABAergic pathway.

Acknowledgement: This research was supported by the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education (NRF-2016R1D1A1B03934176).

Keywords: CA3 hippocampal region, GABAA receptor, Interictal epileptiform activity, Mossy fiber, Zn^{2+}

P-03-025

Simultaneous identification of dose-response curves for ion channels using deep-learning

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Recently, CiPA (comprehensive in vitro pro-arrhythmia assay) becomes a new paradigm for cardiac toxicity assay in the new drug development. For in-silico assay in this protocol, the dose-response curves for each ion channels are required and can be only experimentally obtained using ion channel expressed cell-line. Since CiPA requires the data of the multi-ion channels, the experiments must be time consuming and inherently contain error because ion channel expressed in cell-line is not quite the same as in natural cell. We tested possibility that the dose-response curves for the multi-ion channels can be simultaneously identified in natural cells con-

taining the required ion channels. Firstly, we created the pulse protocol to produce the currents for targeted channels. Using the O'Hara-Rudy (ORd) human ventricular cell model, we applied this protocol with the random conductance of each ion channels and generated 500,000 current data. We trained our deep learning-based conductance prediction model with 400,000 data and tested the model with 100,000 data. Our model can identify the conductance with the correlation coefficient, 0.999 for seven ion channels. And then, using the dose-response data published in CiPA for 11 drugs, we generated the current data for each drug. Our model can show the same dose-response for each ion channels. Our method can considerably accelerate the drug effect identification. For example, if an experiment is performed five times at four different concentrations in each of the seven ion channels, a total of 140 experiments must be performed. Our method only requires 20 experiments in natural cell and can reduce the number of the experiments to 1/(the number of ion channels). And more, we found we can identify the drug effect on the late sodium without ATX II (anemone toxin II) treatment. Our method needs to be verified with the real experiments, however, clearly showed the possibility to reduce the time, effort, and cost and to change the paradigm of new-drug development.

Acknowledgement: This research was supported by a grant (22213MFD5392) from the Ministry of Food and Drug Safety in 2022.

Keywords: Deep-learning, Multi-ion channel assay, Dose-response curve, CiPA, Cardiac electrophysiology

P-03-026

The role of STING-IRF3 signaling in GATs expression and its implications in cognitive functions

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Stimulators of interferon genes (STING), a signaling molecule essential for the transcription of host defense genes type I interferons and proinflammatory cytokines. The role of STING and STING-activated pathways have been studied in systemic inflammation, infection, and cancer but their role in the CNS remains unclear. Here our study uncover that genetic deletion of STING decreased GABA transporters (GATs) activity in the hippocampus, which cause increased tonic GABAA inhibition in dentate gyrus granules (DGG) cells and resulted in learning and memory deficits in mice. We also confirm that the deletion of STING did not affect anxiety and depression-like behavior and motor skill learning in mice. Finally, we confirm that the GATs expression is regulated by tank binding kinase1 (TBK1) and interferon regulatory factor3 (IRF3) activity-dependent manner. Furthermore, our result suggests that IRF3 is a novel transcriptional regulator of GATs. Our study reveals for the first time that the STING-IRF3 signaling pathway is a novel transcriptional linkage between the innate immune pathway and GABA-dependent cognitive deficits.

Keywords: STING, IRF3, GATs, Tonic GABAA inhibition, Memory

P-03-027

CFTR Channel Regulation of Bicarbonate Permeability by WNK1

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An increase in relative HCO_3^- permeability (PHCO3/PCI) of cystic fibrosis transmembrane conductance regulator (CFTR) channel by $[Cl^-]$ -sensitive kinases is crucial for pancreatic HCO_3^- secretion. Defects in pancreatic

HCO₃⁻ secretion due to CFTR mutations are associated with cystic fibrosis and pancreatitis. However, the molecular mechanisms by which [Cl⁻]-sensitive kinases regulate CFTR anion selectivity are elusive. We examined the mechanisms of CFTR PHCO₃/PCI regulation by [Cl⁻]-sensitive kinases using the electrophysiological and molecular approach, including whole-cell, outside-out, inside-out patch-clamp recordings, and molecular dissection of WNK1 and CFTR proteins. The expression of WNK1 alone was sufficient enough to increase the CFTR PHCO₃/PCI in patch-clamp recordings. In the molecular dissection of the WNK1 protein, the WNK1 kinase domain contributes to CFTR PHCO₃/PCI regulation by direct association with CFTR. Based on various results obtained, the CFTR PHCO₃/PCI is regulated by [Cl⁻]_i and a WNK1-dependent mechanism. Defects in this process depict the pathogenesis of some CFTR-related disorders.

Keywords: Cfr, Bicarbonate, Wnk1

P-03-028

In vitro electrophysiological assessment for proarrhythmia risk prediction under CiPA initiative

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The implementation of the International Council on Harmonization (ICH) S7B and E14 regulatory guidelines has been successful in preventing the introduction of potentially arrhythmogenic drugs to the market, but it has also unduly limited drug development by focusing on human ether-a-go-go related gene (hERG) potassium channel block as essential determinants of proarrhythmia risk. In response, the Comprehensive in vitro Proarrhythmic Assay (CiPA) initiative was proposed that integrates multiple cardiac ion channel pharmacology data in vitro into a human induced pluripotent stem cell-derived cardiomyocytes (human iPSC-CMs) model in vitro for proarrhythmia risk prediction. Based on the CiPA initiative, we examined the effect of the 3 CiPA training set drugs categorized as a high (sotalol), an intermediate (chlorpromazine) and a low proarrhythmic risk drug (mexiletine) on in vitro multiple cardiac ion channel assay using the manual patch clamp technique. To further confirm proarrhythmic risk of those drugs, microelectrode array (MEA) was performed in human iPSC-CMs using field potential duration (FPD) recordings. As a result, all of sotalol, chlorpromazine and mexiletine blocked hERG potassium currents, whereas chlorpromazine and mexiletine inhibited Cav1.2 calcium currents more than sotalol, expecting the reduction of QT prolongation and TdP occurrence via balanced ion channel block by chlorpromazine and mexiletine. Corresponding to these results, furthermore, sotalol induced more prolonged FPD in human iPSC-CMs as compared to chlorpromazine and mexiletine. It shows the correlation with the CiPA initiative's validity that a study should be tested the drug effects on Cav1.2 channel and Nav1.5 channels as well as hERG channels. In this study, using the method proposed by the CiPA initiative, it was possible to predict the arrhythmogenic risk groups of drugs more accurately, which could be presented as a new strategy in the cardiac safety and pharmacology assessment.

Acknowledgement: 21181MFD5276.

Keywords: In vitro electrophysiological assay, Patch-clamp assay, MEA, CiPA initiative

P-03-029

Inhibition of voltage-dependent K⁺ channels by antimuscarinic drug fesoterodine in 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 IC₅₀ value of $3.19 \pm 0.91 \mu\text{M}$ 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

P-03-030

Blockade of Kv3.1 by MK801, a PCP-derivative NMDA receptor inhibitor

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MK801, a phencyclidine (PCP) derivative well known as dizocilpine, is a potent noncompetitive antagonist of the N-Methyl-D-aspartate receptor (NMDAR). The NMDAR plays a critical role in mediating excitatory synaptic transmission in the central nervous system (CNS), and this receptor is very important in regulating synaptic plasticity, learning and memory functions. MK801 is known to induce schizophrenia by blocking the NMDAR in animal models. Schizophrenia is a serious mental illness accompanied by fatal cognitive impairment. In addition, several studies have reported that such cognitive impairment is associated with functional defects of ion channels and GABAergic interneurons. The Kv3.1 channels are voltage-gated K⁺ (Kv) channels involved in the rapid repolarization of the action potential in neurons. These channels are richly expressed in GABAergic interneurons and are associated with the generation of fast and repetitive spikes. Decrease of Kv3.1 in CNS has been reportedly associated with schizophrenia. In the present study, the effect of MK801 on Kv3.1 was investigated using a whole-cell patch-clamp technique in Chinese hamster ovary (CHO) cells stably expressing Kv3.1. MK801 caused a concentration-dependent inhibition of Kv3.1, with value of an IC₅₀ of $10.81 \mu\text{M}$ and a Hill coefficient of 0.89. The effect of MK801 on Kv3.1 showed a use-dependent block that induces progressive inhibition by repeated stimulation at increased frequencies (1 Hz and 2 Hz). Consistent with this, recovery from inactivation of Kv3.1 was also delayed in the presence of MK801. Also, MK801 induced a hyperpolarizing shift in the voltage dependence of steady-state inactivation curves of Kv3.1. Taken together, these results indicate that MK801 blocked Kv3.1 expressed in CHO cells in a state-dependent manner. Given the importance of Kv3.1 in fast-spiking GABAergic interneurons, our findings suggest the possibility that MK801 may affect the firing patterns of action potential in inhibitory

neurons, triggering the onset and symptoms of schizophrenia.

Keywords: Kv3.1, MK801, Schizophrenia, NMDA receptor, Fast-spiking GABAergic interneuron

P-04-001

Vasodilation by trelagliptin, a DPP-4 anti-diabetic drug, via activation of Kv channels and SERCA pumps 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 Ca²⁺-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 Ca²⁺ 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

P-04-002

Vasodilatory effect of antidiabetic omarigliptin by activating Kv Channels and SERCA pump 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 Ba²⁺, ATP-sensitive K⁺ channel inhibitor glibenclamide, and large-conductance Ca²⁺-activated K⁺ channel inhibitor paxilline did not alter its vasodilation. Pretreatment with the sarco/endoplasmic reticulum Ca²⁺-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 Ca²⁺-activated K⁺ channel inhibitor apamin, together with the intermediate-conductance Ca²⁺-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

P-04-003

A novel regulator of skeletal muscle functions

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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 Ca²⁺-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. Ca²⁺ movement in the myotubes was examined using single-cell Ca²⁺ imaging. Unlike wild-type (WT) TRIM32, NHL-Del did not enhance the amount of Ca²⁺ release from the sarcoplasmic reticulum (SR), Ca²⁺ release for excitation-contraction (EC) coupling. TRIM32 may participate in the regulation of Ca²⁺ movement for skeletal muscle contraction. Functional defects in TRIM32 due to mutations in NHL repeats may be pathogenic toward LGMD2H.

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

P-04-004

Calsequestrin 1 Is an Active Partner of Stromal Interaction Molecule 2 in Skeletal Muscle

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Calsequestrin 1 (CASQ1) in skeletal muscle buffers and senses Ca²⁺ in the sarcoplasmic reticulum (SR). CASQ1 also regulates store-operated Ca²⁺ 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 Ca²⁺ imaging experiments and transmission electron microscopy observations. The CASQ1-STIM2 interaction via the C1 region decreased SOCE, increased intracellular Ca²⁺ release for skeletal muscle contraction, and changed intracellular Ca²⁺ distributions (high Ca²⁺ in the SR and low Ca²⁺ in the cytosol were observed). Furthermore, the C1 region itself (which lacks Ca²⁺-buffering ability but has STIM2-binding ability) decreased the expression of Ca²⁺-related proteins (canonical-type transient receptor potential cation channel type 6 and calmodulin 1) and induced mitochondrial shape ab-

normalities. 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, SOCE, STIM2, Skeletal muscle

P-04-005

Ultra-weak light emission improves mitochondrial respiration in heart and skeletal muscle of mice

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Mitochondria are important bioenergetic and biosynthetic factories for cellular function and human health. Ultra-weak light emission plays an essential role for immune system and metabolic function. However, the role of ultra-weak light emission in mitochondrial respiration is largely unknown yet. Here, we evaluated the effect of 8-week of ultra-weak light emission on mitochondrial respiration in heart and skeletal muscle of mice. The mice were assigned to the following two groups: morning ultra-weak light emission (ME, n = 6) and evening ultra-weak light emission (EE, n = 6). After 8 weeks, we measured the mitochondrial respiration in heart and skeletal muscle. Mitochondrial respiration of heart and skeletal muscle was higher EE group than ME group. This is the first evidence of beneficial actions of ultra-weak light emission on mitochondrial respiration in heart and skeletal muscle of mice. These findings suggest ultra-weak light could be an attractive strategy for mitochondrial function as a multiple therapeutic target with metabolic diseases.

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Keywords: Mitochondria, Ultra-weak light emission, Heart, Skeletal muscle

P-04-006

Fetuin-B ameliorates dexamethasone-induced atrophy in C2C12 mouse skeletal muscle cells

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Skeletal muscle atrophy can be caused by a variety of conditions, including aging, disuse, denervation, malnutrition and several diseases and is characterized by reduction in protein content, muscle fiber size, and force. Skeletal muscle atrophy can impair exercise capacity and quality of life. However, there are still no effective drugs or treatments for muscle atrophy. Dexamethasone, an inducer of skeletal myofiber atrophy, has been used for many muscle atrophy studies. Fetuin-B has been reported to be involved in diverse responses such as plaque instability, vascular inflammation, glucose and lipid metabolism, and the occurrence of diabetes, but it is not yet known whether fetuin-B affects muscle atrophy. In the present study, we investigated the effects of fetuin-B on skeletal muscle cell atrophy and explored the underlying possible mechanisms. C2C12 mouse skeletal myoblasts were differentiated into myotubes. Myotube atrophy was induced by dexamethasone. The diameter of myotubes was measured under a light microscope and protein expression was analyzed by western blots. Fetuin-B did not affect the viability in C2C12 cells. Dexamethasone, a corticosteroid atrophic factor, diminished the myotube diameters, which was reversed

in myotube treated with fetuin-B. Treatment with fetuin-B resulted in reduction of the elevated MuRF-1 expression and p38 MAPK in C2C12 myotubes exposed dexamethasone. In addition, fetuin-B also reversed the decreased phosphorylation levels of AKT and mTOR in myotubes in response to dexamethasone. These findings suggest that fetuin-B may ameliorate dexamethasone-induced C2C12 myotube atrophy, probably by regulating signaling proteins such as MuRF-1, p38 MAPK, AKT and mTOR. Therefore, fetuin-B may be a potential therapeutic molecule for the amelioration or treatment of skeletal muscle atrophy and its related disorders. However, further studies are needed to clarify the effect of fetuin-B on muscle atrophy in vivo.

Acknowledgement: This work has supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (No. NRF-2020R1A2C1101901).

Keywords: Skeletal muscle, Muscle atrophy, Fetuin-B, Dexamethasone, Myotubes

P-05-001

miR204 potentially promotes non-alcoholic fatty liver disease by inhibition of cpt1a in mouse hepatocytes

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Non-alcoholic fatty liver disease (NAFLD) is associated with hepatic metabolism dysfunction. However, the mechanistic role of miR204 in the development of NAFLD is unknown. We investigate the functional significance of miR204 in the evolution of NAFLD. IDH2 KO mice fed a normal diet (ND) or HFD increased body weight, epididymal fat-pad weight, lipid droplet in liver, blood parameter and inflammation compared to WT mice fed a ND or HFD. Moreover, the expression of miR204 is increased in mice with IDH2 deficiency. Increased miR204 by IDH2 deficiency regulates carnitine palmitoyl-transferase 1a (cpt1a) synthesis, which inhibits fatty acid β -oxidation. Inhibition of miR204 prevents the disassembly of two fatty acid-related genes by activating CPT1a expression, which decreases lipid droplet in liver, inflammatory cytokines, epididymal fat pad weight, blood parameters. Increased miR204 by IDH2 deficiency promotes the pathogenesis of HFD-induced NAFLD by regulating hepatic fatty acid metabolism and inflammation.

Keywords: Non-alcoholic fatty liver disease, MiR204, CPT1a, High Fat Diet, IDH2

P-05-002

DPP-4 inhibitor prevents cardiomyopathy via improvement of mitochondrial function and reduction of cardiac fibrosis in type 2 diabetic mice

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Dipeptidyl peptidase-4 (DPP-4) inhibitors are popularly used antihyperglycemic drugs for the treatment of type 2 diabetes mellitus (T2D). Currently, the pleiotropic effects of DPP-4 inhibitors have drawn much attention. Our investigation aimed to examine whether evogliptin, a recently developed DPP-4 inhibitor, could protect against T2D-induced cardiomyopathy. Eight-week-old diabetic and obese db/db mice received evogliptin treatment (100mg and 300mg/kg/day), db/db control mice and db/m control mice received with equal amounts of vehicle daily for 12 weeks. Body weight and feeding weight was measured every week. Cardiac function was as-

sessed using echocardiography at before and after feeding 12 weeks. Histological and molecular markers of cardiac fibrosis were assessed in the left ventricle (LV) at 20 weeks old. The results showed that evogliptin improved T2D-induced cardiac dysfunction, as shown by analysis of 2D and doppler echocardiography (LV ejection fraction, fractional shortening was higher, E/A and e'/a' ratios increased, E/e' ratio and deceleration time decreased in evogliptin - treated groups compared to db/db control group). Evogliptin also attenuated interstitial fibrosis and reduced mitochondrial damage. Our data suggest that evogliptin might be of benefit in cardio-dysfunction patients with type 2 diabetes.

Acknowledgement: This work was supported by the National Research Foundation of Korea and the Ministry of Education of Korea [NRF-2020R1A4A1018943, and NRF-2018R1A2A3074998].

Keywords: DPP-4 inhibitor, Diabetic cardiomyopathy, Mitochondria

P-05-003

Long-term exposure of ethylenethiourea induces nephrotoxicity in male C57BL/6 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 poly-cystogenesis 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, an 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.

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Keywords: Ethylenethiourea, Polycystic kidney disease, Nephrotoxicity, MiR-17~92 cluster

P-05-004

Functional analysis of novel SCN5A mutations related to Brugada syndrome

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Brugada syndrome (BrS) is an arrhythmogenic disorder that has been linked to mutations in SCN5A, the gene encoding for the pore-forming α -subunit of the cardiac Na⁺ channel. Recently, novel SCN5A missense mutations were identified in a BrS patient in Korea; A385T and R504T in the loop connecting transmembrane segments 5 and 6 in domain 1 (S5-S6 in DI) and segments 6 and 1 between domain 1 and 2 (DI-DII linker), respectively. To elucidate the mechanism of the BrS phenotype, we performed to whole-cell patch clamp and immunoblot assay in a heterologous expression system. The wild-type (WT) and mutant SCN5A were transiently transfected in HEK293 cells. WT, A385T, R504T, and double mutant (A385T/R504T) showed no significant differences in the current density and the voltage-dependent activation. However, when co-transfected with β -subunit SCN1B, the current densities of R504T and A385T/R504T were significantly suppressed while that of A385T was not affected. The recovery from inactivation of all mutants were slower than that of WT. The immunoblot assay showed no difference in the expression of both total and surface protein of WT and double MT(A385T/R504T) even with β -subunit SCN1B. These results suggest that the α -subunit (A385T/R504T) mutation induces the BrS phenotype due to functional impairment despite the preserved protein expression. In particular, the R504T mutation among the two mutations(A385T/R504T) is mainly responsible for the pathophysiological mechanism.

Keywords: Brugada syndrome, NaV1.5, Mutation, B-subunit, SCN1B

P-05-005

Role of Rho-associated kinase (ROCK) in the different speed of relaxation between pulmonary arteries and mesentery arteries of rats

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Pulmonary arteries (PA) are exposed to hemodynamic environment and contractile stimuli different from those of systemic arteries. Here, we investigated the relaxation speed and the in vitro effects of NO-dependent signaling modulators to compare between isolated PA and mesenteric arteries (MA) of rats. In the dual-wire myography study, PA showed significantly slower recovery of relaxation after the contractile response to 80 mM KCl (80K) or to pharmacological agonists (U46619 and PGF2a). Surprisingly, addition of 30 μ M SNP could not prevent the 80K contraction in PA while significantly attenuated the contraction of MA. The pretreatment with soluble guanylate cyclase (sGC) inhibitor (ODQ, 10 μ M) markedly further slowed the relaxation of PA after 80K or agonist-induced contraction. Perplexingly, immunoblot assay revealed higher expression of sGC- α with similar level of sGC- β 1 in PA than MA. In contrast, the expression of RhoA-kinase (ROCK) was higher while myosin light chain phosphatase (MYPT) appeared lower in PA than MA. The treatment with ROCK inhibitor (Y27632, 10 μ M) could abolish the different of relaxation response between PA and MA. Consistent with these results, phosphorylation of myosin light chain 2(MLC2) was higher in PA than MA. Taken together, we speculate that despite the higher expression and the functional importance of NO/sGC pathway in PA, enhanced smooth muscle contraction via MLC2 diphosphorylation and the higher level of ROCK, relatively low MYPT in PA are responsible for the characteristic slow relaxation after removal of stimuli.

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Republic of Korea (grant no.NRF2021R1A2C200724311).

Keywords: Pulmonary artery, Mesenteric artery, ROCK, Relaxation

P-05-006

BH4 activates CaMKK2 and rescues the cardiomyopathic phenotype in rodent models of diabetes

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Diabetic cardiomyopathy (DCM) is a major cause of mortality/ morbidity in DM patients. Although tetrahydrobiopterin (BH4) shows therapeutic effect on myocardial cells and mitochondria in DCM, the underlying mechanisms remain unknown.

We determined the involvement of BH4 deficiency in DCM and the therapeutic potential of BH4 supplementation in a rodent DCM model.

A decreased BH4: total biopterin ratio in heart and mitochondria accompanied by cardiac remodeling, lower cardiac contractility, and mitochondrial dysfunction. Prolonged BH4 supplementation improved cardiac function, corrected morphological abnormalities in cardiac muscle, increased mitochondrial activity. Oxidative phosphorylation (OXPHOS) as the BH4-targeted biological pathway in diabetic hearts. BH4 bound to calcium/calmodulin-dependent protein kinase kinase 2 (CaMKK2) and activated downstream AMP-activated protein kinase/cAMP response element binding protein/PGC-1 α signaling to rescue mitochondrial and cardiac dysfunction in DCM. These results suggest BH4 as a novel endogenous activator of CaMKK2.

Keywords: BH4, CaMKK2, Cardiomyopathy, Diabetes

P-05-007

Integrin $\alpha\text{v}\beta 3$ alteration by fluid shear stress in podocyte

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Glomerular hyperfiltration is a key risk factor for the progression of glomerular diseases. Podocyte, a gatekeeper of the glomerular filtration barrier, is exposed to fluid shear stress whose overactivation is responsible for disruption of cell-matrix interactions leading to glomerular disease. Integrins (Itg) play a pivotal role in not only cell-matrix interaction but also in sensing the mechanical forces by fluid shear stress. However, the cell type-specific expression and function of Itg in cell-matrix interaction and the underlying mechanism of fluid shear stress-induced podocytopathy are still poorly understood. Here we examined whether fluid flow shear force causes morphological and functional changes in human podocytes. Fluid flow shear stress (FFSS) was applied in short-term (30 mins) and long-term (up to 7 days) periods with different extracellular matrix (ECM) conditions, such as collagen type I, IV, and fibronectin (FN). FFSS upregulated the activated form of Itg $\alpha\text{v}\beta 3$ with actin cytoskeletal remodeling and morphological changes in human podocytes. FFSS increased uPAR and FN protein expression supporting the notion that Itg $\alpha\text{v}\beta 3$ activation with FN is a downstream effector of uPAR activation under mechanical stress conditions. The FN-coated condition promoted cell viability against applying FFSS demonstrating that proper cell-matrix interaction is vital for adaptation and survival of human podocyte FFSS. Taken together, this study demonstrated the activation of

Itg $\alpha\text{v}\beta 3$ by uPAR leads to the upregulation of FN in human podocytes. This may improve the physical affinity of podocyte and GBM to protect podocyte detachment from GBM by FFSS. Our findings provide new insights for therapeutic approaches to glomerular disease and treatment.

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Keywords: Integrin $\alpha\text{v}\beta 3$ (Itg $\alpha\text{v}\beta 3$), Fluid shear stress (FSS), Podocyte, Glomerular disease, Cell adhesion

P-05-008

Echinochrome A reverses kidney abnormality and reduces blood pressure in a rat model of preeclampsia

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We aimed to observe the effects of Echinochrome A (Ech A) on structural changes of systemic organ using a rat model of preeclampsia. Our results showed that infusion of Angiotensin II (Ang II) through osmotic pump (1ug/kg/min) on the 8th day of pregnancy increased systolic/diastolic blood pressure, reduced fetal weight, fetal crown-rump length and placental weight, disrupted kidney the diameter and capillary of the glomerulus. Ech A treatment on the 14 GD (100ug/ul) through jugular vein reduced systolic/diastolic blood pressure on 20 GD, reversed glomerulus alterations but did not affect fetal weight, fetal crown-rump length, placental weight. No change was observed in the heart and liver in Ang II group but uterus showed abnormality. Ech A only partly reversed the effect on uterus. mRNA expressions of TNF- α was increased, (IL)-10 and VEGF were reduced all four tissues tested in Ang II group and Ech A restored the 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. We suggest that Ech A modulates inflammation and apoptosis in systemic organs in Ang II-induced preeclampsia and preserves kidney structure and reduces blood pressure.

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Keywords: Angiotensin II, Apoptosis, Blood pressure, Echinochrome A, Kidney, Preeclampsia, TNF- α

P-05-009

TRPC6 deficiency causes adipocyte dysfunction and obese-like phenotypes

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Deregulation of calcium signaling is a key feature of various metabolic disorders including obesity and diabetes. Here, we reported that TRPC6 deficiency disturbed lipid metabolism in adipose tissues leading to obesity. TRPC6 deficient (TRPC6 KO) mice showed higher body weight, decreased respiratory quotient, oxygen consumption, locomotor activity, and heat

production than wild-type mice. Although TRPC6 KO mice have an obese phenotype, these mice have a consistently lower food intake compared to that of wild-type mice demonstrating that the obese phenotype did not result from food intake. TRPC6 KO mice also have lipid accumulation and infiltration in multiple organs, including brown adipose tissue (BAT), liver, and skeletal muscle (SKM). Furthermore, TRPC6 KO mice have developed insulin resistance and glucose intolerance when challenged with insulin tolerance and glucose tolerance tests.

The occurrence and development of obese phenotypes in TRPC6 KO mice have a tissue- and age-dependent manner. The white adipose tissue (WAT) and BAT were primarily affected with reduced integrity and enlarged adipocytes. TRPC1 and 6 are the dominant TRPC members expressed in the murine pre-white adipocyte. Pre-white adipocytes isolated from TRPC6 KO mice showed reduced differentiation capacity resulting in less mature fat cell formation with a lower oxygen consumption rate and extracellular acidification rate. Perturbation of lipid metabolism by TRPC6 deficiency in adipose tissues may be the primary cause of systemic lipid overload in insulin-target tissues and changes in metabolic phenotypes. This study provides evidence linking adipocyte function, adipogenesis, and Ca^{2+} signaling through the TRPC6 channel. This study was supported by the National Research Foundation of Korea (NRF-2017R1A5A2015369 & NRF-2022R1A2C2011079).

Keywords: TRPC6, Adipogenesis, Obesity, Lipid metabolism, Ca^{2+} signaling

P-05-010

TRPC6 deficiency causes hepatosteatosis through deregulation of adipocyte lipid handling

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The liver is the central organ for lipid metabolism. In previous observation, the *Trpc6* deficient (TRPC6 KO) mice have developed an obese phenotype with higher body weight, and lipid accumulation in multiple tissues including the liver. The expression level of *Trpc6* in mouse liver is relatively low and the pile-up of fat only appeared after adipose tissue hypertrophy at a younger age. Hence, hepatosteatosis in TRPC6 KO mice may be a consequence of adipose tissue malfunction. However, it remains unclear how the liver carries out its function and cross-talks with other metabolic tissues under the stress of TRPC6 KO. TRPC6 KO mice showed a significantly lowered response to insulin as well as glucose disposal after injection. Hepatic insulin pathways were blunted in TRPC6 KO mice. TRPC6 KO liver showed disrupted cristae structures and lower expression of β oxidation genes including acyl-CoA dehydrogenase very long chain (ACADVL) and carnitine palmitoyl transferase 1A (CPT1A). These features may be mediated not only by lipid overload in the liver but also by the loss of liver – adipose tissue communication due to the reduction of adipokines such as fibroblast growth factor 21 (FGF21) or adiponectin (ADPN). In TRPC6 KO mice, FGF21 level was marginally reduced in the liver. On the other hand, ADPN was expressed lower in the adipocyte of TRPC6 KO. Taken together, this study demonstrates that TRPC6 deficiency resulted in hepatic steatosis by the dysregulation of adipose tissue function and provides clues for the molecular mechanism of Ca^{2+} -mediated hepatosteatosis and context-specific systemic lipid handling. This study was supported by the National Research Foundation of Korea (NRF-2017R1A5A2015369 & NRF-2022R1A2C2011079).

Keywords: TRPC6, Steatosis, Lipid metabolism, Adipokine

P-05-011

Maintaining integrity of hair follicles by 3-dimensional co-culture of hair follicles and dermal fibroblast spheroids in collagen hydrogels

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The hair follicle, a skin appendage, is a complex and dynamic mini-organ that undergoes a lifelong cycle of degeneration and regeneration. Aberrant hair cycling causes hair loss, thus hair follicle is a key target for drug development to treat hair loss. In vitro organ culture models of hair follicles as alternatives to animal testing need to be developed for drug testing as well as elucidating hair follicle biology. With an aim to develop a more physiologically relevant in vitro culture model, we developed a hydrogel-based 3D co-culture model of mouse vibrissae hair follicle with dermal fibroblast spheroids. Vibrissae hair follicles were isolated from 2-week-old CD1 mice and cultured either in traditional fluid condition or in 3D collagen hydrogels with or without dermal fibroblast spheroids. After 2 week-culture of hair follicles, the lengths of hair shafts and follicles were measured and the integrity of hair follicles was assessed by histology and immunofluorescence. The lengths of hair shafts increased regardless of in vitro hair follicle culture conditions until 2 weeks. The growth rate was faster in fluid culture than 3D hydrogel culture, which was a pseudo effect due to the upward sliding of hair shaft detached from hair bulb in fluid culture model. In 3D hydrogel environment, co-culture of hair follicles with dermal fibroblast spheroids not only increased the growth rate of hair shafts but also reduced the contraction of hair follicles compared to culture alone in hydrogels. After 2 weeks of culture, hair follicles cultured in fluid medium were disintegrated, and Ki-67 positive proliferating cells as well as Lgr-5, K-15 and CD34 positive stem cells were barely identified. However, in 3D hydrogel environment, the integrity of hair follicles was well maintained and the number of proliferating cells as well as stem cells were more abundant in co-culture of hair follicles with dermal fibroblast spheroids than culture alone in hydrogels. Taken together, 3D co-culture of hair follicles and dermal fibroblast spheroids in collagen hydrogels permits hair follicles to maintain their integrity and proliferating potential in a more physiologically relevant environment via epidermal-mesenchymal interactions, and enables long-term in vitro culture of hair follicles.

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Keywords: Vibrissae hair follicles, Collagen hydrogels, 3D co-culture, Dermal fibroblasts, Hair follicle stem cells

P-05-012

ROS-mediated feedforward upregulation of TRPC6 initiates hepatic stellate cell activation and fibrosis

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The activation of hepatic stellate cells (HSCs) is the primary event of hepatic fibrosis. Reactive oxygen species (ROS) production by injury and disturbances of Ca^{2+} signaling have been implicated in an early stage in HSCs activation leading to fibrosis. Hepatic fibrosis mediators including TGF β and endothelin-1 (ET-1) are not only linked to Ca^{2+} signaling but also po-

tent oxidative stress inducers. It is unclear yet known how injury-produced ROS initiates HSCs activation through Ca^{2+} channels. Bile duct ligation (BDL) and thioacetamide (TAA) administration induced physical and chemical hepatic fibrosis, respectively. Both hepatic injuries stresses BDL and TAA are known to generate mitochondrial ROS. Previously, we identified that the TRPC6 channel is the predominant Ca^{2+} influx mechanism in HSCs activation. TRPC6 and fibrosis markers expressions were increased during HSC activation. H₂O₂ directly upregulated TRPC6 current density and Ca^{2+} influx supporting feedforward TRPC6 activation by ROS. Conversely, TRPC6 activation by OAG and/or ET-1 caused depolarization of mitochondrial membrane potential followed by mitochondrial ROS production. Trpc6 deficiency ameliorated fibrosis induced by TAA or BDL in mice. Moreover, ROS was significantly elevated in both fibrosis animal models and it was reduced by Trpc6 knockout. Fibrosis marker expressions were reduced by suppressing TRPC6 in vitro primary and cultured HSCs. In conclusion, our findings demonstrate that ROS-mediated feedforward upregulation of TRPC6 initiates hepatic stellate cell activation and their positive feedback signaling pathway accelerates hepatic fibrosis. These results provide a new perspective on the pathogenesis of hepatic fibrosis and clues for potential therapeutics for cirrhosis. This study was supported by the National Research Foundation of Korea (NRF-2017R1A5A2015369 & NRF-2022R1A2C2011079).

Keywords: Oxidative stress, Hepatic fibrosis, TGF β , TRPC6

P-05-013

Hidden re-initiation of transcription in a KCNH2 frameshift mutation (c.453delC) produces impaired hERG K⁺ channels and the heterozygote patient-derived iPSC-CMs show LQT phenotype

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Patient-specific cardiomyocytes from human induced pluripotent stem cells (hiPSC-CMs) are valuable for studies in inherited cardiac diseases. A recent study reported single nucleotide C deletion mutation in the exon 3 of KCNH2 gene (c.453delC-KCNH2, p.151Pfs +15X in hERG) associated with LQT syndrome (Park JK et al., 2013). Since the 453delC-KCNH2 resulted the frameshift of the coding sequences, a premature termination of translation at the N-terminal region was suggested. However, there is an additional initiation codon next to the mutated residue. To elucidate the precise mechanism of LQT phenotype, we performed whole-cell patch clamp and immunoblot assay in 453delC-KCNH2 hiPSC-CMs and HEK293 cells transfected with 453delC-KCNH2. The 453delC-KCNH2 hiPSC-CMs showed significantly prolonged action potential duration (APD) and reduced density of the rapidly activating delayed rectifier K⁺ current (I_{Kr}). The density of I_{hERG} in HEK293 cells transfected with 453delC-KCNH2 was 10 % of the wild type (WT) I_{hERG}. However, voltage dependence of activation, voltage dependence of inactivation, and deactivation kinetics of 453delC-KCNH2 were not significantly different from those of WT. To study the interaction between WT and mutant, the equimolar amounts of WT and 453delC cDNA were transfected into HEK293 cells. The current density of WT/453delC channels was half of that from the WT channel alone, indicating insignificant dominant negative effect. Immunoblot analysis of WT channel showed 150 kDa of core-glycosylated form and 180 kDa of fully-glycosylated channel. Interestingly, 453delC-KCNH2 overexpressed cells showed 135 kDa and 160kDa suggesting that the translation of shorter form, i.e. N-terminal truncated hERG, actually occurred with subsequent glycosylation. Nevertheless, the markedly reduced I_{hERG} and the prolonged APD indicated functionally impaired state of 453delC-KCNH2, consistent with the LQT2 phenotype.

Keywords: Human induced pluripotent stem cells-cardiomyocyte, Long QT syndrome type 2, KCNH2 mutation

P-05-014

Capsanthin Prevents Atherosclerosis and Vascular Inflammation in ApoE^{-/-} mice

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Capsanthin is one of the major carotenoids of red paprika (*Capsicum annuum* L.). Capsanthin is regarded as antioxidant functioning and anti-inflammatory agents for humans. However, its role in atherosclerosis is yet to be fully elucidated. This study investigated the role of dietary capsanthin in vascular inflammation in atherosclerotic mice. We assessed the anti-atherosclerotic effects of daily oral administration of capsanthin (0.5 mg/kg of body weight/day) in apolipoprotein E-deficient (ApoE^{-/-}) mice fed a Western-type diet (WD). Capsanthin treatment suppressed vascular cell adhesion molecule 1 expression and nuclear factor- κ B ser536 phosphorylation in tumor necrosis factor- α -stimulated cultured endothelial cells. Dietary capsanthin significantly inhibited the WD-induced elevation in the plasma levels of total cholesterol, low-density lipoprotein cholesterol (LDL-C), and triglyceride in mice. Interestingly, capsanthin reduced aortic plaque formation and VCAM-1 expression, which is vascular inflammation, in atherosclerotic mice. In addition, the neutrophil-lymphocyte ratio, a systemic inflammatory marker, was inhibited in capsanthin-treated mice. Furthermore, capsanthin significantly reduced the levels of proinflammatory cytokines, such as TNF- α , interleukin-6, and monocyte chemoattractant protein-1, in the plasma of atherosclerotic mice. Collectively, our data demonstrate that dietary capsanthin plays a protective role against atherosclerosis in hyperlipidemic mice. This protective effect could be attributed to the anti-inflammatory properties of capsanthin.

Keywords: Capsanthin, Atherosclerosis, Vascular inflammation

P-06-001

Mitochondrial modulation protects blood-brain barrier integrity by increasing junctional protein expression in cerebrovascular cell

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Blood-brain barrier (BBB) is composed of endothelial cells, astrocytes, and pericytes. 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 the 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. Although the importance of mitochondria in cerebral endothelial cells is already known for BBB integrity, therapeutic agents for the maintenance of BBB and the prevention of BBB disruption still need to be developed. Previously, we reported that mitochondrial defect closely correlation of BBB disruption by loss of tight junction. In this study, we found the supplement which can modulate mitochondrial function before the induction of oxidative stress by oxygen-glucose deprivation (OGD) in the cerebral endothelial cell line. Junctional proteins are investigated after OGD, ischemic condition with the supplement pretreatment. Furthermore, we examined mitochondrial respiration and mitohormetic response to investigate the effect of the supplement on mitochondrial functions. Pretreatment of the supplement in mouse cerebral endothelial cells increased the junctional protein expression accompanying mitochondrial modulation. These results demonstrate that modulating mitochondria in cerebral endothelial cells can prevent BBB

disruption.

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Keywords: Mitochondria, Blood-brain barrier, Endothelial cell, Stroke

P-06-002

Reduced branched-chain aminotransferase activity alleviates metabolic vulnerability caused by dim light exposure at night in *Drosophila*

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The rhythmic pattern of biological processes controlled by light over 24 h is termed the circadian rhythm. Disturbance of circadian rhythm due to exposure to light at night (LAN) disrupts the sleep-wake cycle and can promote cardiovascular disease, diabetes, cancer, and metabolic disorders in humans. We studied how dim LAN affects the circadian rhythm and metabolism using male *Drosophila*. Wild-type flies exposed to the dim light of 10 lux at night displayed altered 24 h sleep-wake behavior and expression patterns of circadian rhythm genes. In addition, the flies became more vulnerable to metabolic stress, such as starvation. Whole body metabolite analysis revealed decreased amounts of branched-chain amino acids (BCAAs), such as isoleucine and valine. The dim light exposure also increased the expression of branched-chain amino acid aminotransferase (BCAT) and branched-chain α -keto acid dehydrogenase (BCKDC) enzyme complexes that regulate the metabolism of BCAAs. Flies with the Bcat heterozygous mutation were not vulnerable to starvation stress, even when exposed to dim LAN, and hemolymph BCAA levels did not decrease in these flies. Furthermore, the vulnerability to starvation stress was also suppressed when the Bcat expression level was reduced in the whole body, neurons, or fat body during adulthood using conditional GAL4 and RNA interference. Finally, the metabolic vulnerability was reversed when BCAAs were fed to wild-type flies exposed to LAN. Thus, short-term dim light exposure at night affects the expression of circadian genes and BCAA metabolism in *Drosophila*, implying a novel function of BCAAs in suppressing metabolic stress caused by disrupted circadian rhythm.

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Keywords: Circadian rhythm, Branched-chain amino acids, Branched-chain aminotransferase, Metabolic stress, *Drosophila*

P-06-003

Empagliflozin prevents diabetic cardiomyopathy by attenuating cardiac lipotoxicity in type 2 diabetic db/db mice

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Diabetic cardiomyopathy (DCM) is one of the major causes for end stage heart failure (HF), which brings about mortality and morbidity in type 2 diabetes mellitus patients. Empagliflozin (EMPA), a sodium-glucose cotransporter 2 inhibitor (SGLT2i), showed cardioprotective effects against DCM but the molecular mechanism remains unclear. We investigated whether EMPA prevents cardiac dysfunction by reducing lipotoxicity in obese db/db mice. Male C57BL/6J mice were set as the control and male db/db mice

were treated with or without EMPA (10 mg/kg/day) for up to 10 weeks. Treated db/db mice with EMPA reduced body weight and blood glucose level. EMPA improved both systolic and diastolic functions of db/db mice. EMPA reduced fatty acid uptake of cardiomyocytes by attenuating the expression of CD36 leading to decrease triglyceride accumulation and alleviating allosteric control of mitochondrial fatty acid oxidation. In addition, EMPA treatment rescued cardiac mitochondrial damage and improved its function. Taking together, the data suggest that EMPA might be of benefit in cardiac lipotoxicity patients with type 2 diabetes and obesity.

Keywords: Diabetic cardiomyopathy, SGLT2 inhibitor, Empagliflozin, Lipotoxicity

P-06-004

Activation of Sarco/Endoplasmic Reticulum Ca^{2+} ATPase Increases Mitochondrial Biogenesis and Protects Pancreatic β -cells from Lipotoxicity

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High Ca^{2+} level in the ER, developed by sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA), is critically required for protein folding and signal generation. On the other hand, excessive Ca^{2+} release or decreased SERCA's activity induces unfolded protein accumulation and ER stress in pancreatic β -cell. However, consequences of enhancing ER Ca^{2+} uptake on β -cell survival and functions have not been observed. A small molecule SERCA activator, CDN1163, increases insulin synthesis and exocytosis from mouse islets. In sorted β -cells, cytosolic Ca^{2+} oscillation was more sensitized to glucose stimulation and potentiated by CDN1163. CDN1163 augmented ER and mitochondrial Ca^{2+} contents, biogenesis, membrane potential, respiratory activities, and ATP synthesis. Overexpression of SERCA2 recapitulated the CDN1163's effects, while knockdown of SERCA2 abolished the stimulatory actions of CDN1163. Notably, CDN1163 incubation prevented ER Ca^{2+} depletion, mitochondrial dysfunction, secretory defects, and apoptotic death in palmitate-treated β cells. These results suggest that targeting SERCA activities could be an effective therapeutic strategy for β -cell lipotoxicity and type 2 diabetes.

Keywords: Sarco/endoplasmic reticulum Ca^{2+} ATPase, Pancreatic β -cell, Insulin secretion, Mitochondria, Lipotoxicity

P-06-005

Peri-lysosomal Calcium Overload by Palmitate in Pancreatic β -cells

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Saturated fatty acids are known to induce lipotoxicity in pancreatic β -cells. Pathogenic mechanisms including defective autophagy have been proposed; however, the mechanism underlying the impairment remains unclear. We identified the association between mTORC1 and TRPML1 channel which contributed to autophagic inhibition in palmitate-treated pancreatic β -cells. Palmitate exposure induced oxidative stress and ER Ca^{2+} depletion, followed by an elevated peri-lysosomal Ca^{2+} that led to decreased autophagic flux. Palmitate upregulated mTORC1 signaling which was Ca^{2+} /Calmodulin dependent. Simultaneously, TRPML1-mediated lysosomal Ca^{2+} release was abolished by palmitate or mTORC1 activator. Selective inhibi-

tion of mTORC1 by Torin-1 restored the activity of TRPML1 and autophagic flux. Furthermore, there have been links between extracellular Ca^{2+} , ROS generation, ER stress, and mTORC1 activation. Mitochondrial ROS scavenger (mitoTEMPO), Ca^{2+} channel blocker (Verapamil), and SERCA activator (CDN1163) have shown protective effects on autophagic suppression in palmitate-treated β -cells. Taken together, our data suggest that mTORC1 and TRPML1 play a critical role in defective autophagy of β -cell lipotoxicity. Restoring peri-lysosomal Ca^{2+} homeostasis could be a novel therapeutic strategy against metabolic diseases.

Keywords: Peri-lysosomal Ca^{2+} , Lipotoxicity, Pancreatic β -cells, MTOR, TRPML1

P-06-006

Loss of SCAP induces obesity through shifting macrophage polarization in adipose tissue

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Sterol regulatory element binding protein (SREBP) cleavage-activating protein (SCAP) plays a critical function in regulating triglyceride and cholesterol synthesis in the body. Although SCAP regulates lipid metabolism in metabolic tissues, such as the liver and muscles, the effect of macrophage-specific SCAP deficiency in adipose tissue macrophages (ATMs) of patients with metabolic diseases is not completely understood. Here, we examined the function of SCAP in high-fat/high-sucrose diet (HFHS)-fed mice and investigated its effects on the polarization of classical activated macrophages in adipose tissue. Macrophage-specific SCAP knockout (mKO) mice were generated through crossbreeding lysozyme 2-cre mice with SCAP floxed mice and fed HFHS for 12 weeks. Primary macrophages were derived from bone marrow cells. We found that fat accumulation increased in HFHS-fed SCAP mKO mice, accompanied with the polarization of classical activated macrophages in adipose tissue. Furthermore, lipopolysaccharide-mediated SREBP-1a activation upregulated cholesterol 25-hydroxylase transcription, resulting in an increase in the production of 25-hydroxycholesterol (25-HC), an endogenous agonist of liver X receptor alpha (LXR α). In addition, SCAP deficiency stimulated M1 macrophage polarization via suppression of cholesterol efflux by reducing 25-HC-dependent LXR α activation in macrophages. Overall, the activation of the SCAP-SREBP-1a complex in macrophages may provide a novel therapeutic strategy that ameliorates obesity by controlling cholesterol homeostasis in ATMs.

Acknowledgement: This work was supported by an NRF grant funded by the Korea Government (MSIP) [NRF-2021R1A4A1029238].

Keywords: SCAP, White adipose tissue, Macrophages, Cholesterol 25-hydroxylase, Cholesterol efflux

P-07-001

Humanin and formylated Humanin promote skin wound healing through the STAT3 signaling pathway

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Mitochondrial dysfunction is a hallmark of aging and age-related diseases.

Open reading frames in circular mitochondria DNA encode several micropetides, called mitochondrial-derived peptides (MDPs) which are known to be associated with aging and age-related diseases. However, MDPs and their mechanisms of action in skin tissue are poorly understood. Here, we examined that two MDPs, humanin (HN) and its formylated form (f-HN), play a crucial function in promoting wound healing. Wounding was performed on the in vivo skin-hairless mouse model. HN and f-HN were applied through cutaneous injection and followed for 8 days after the wounding. Normal human epidermal keratinocytes (NHEK cells) were used to conduct in vitro experiments. Interestingly, both HN and f-HN treatments significantly reduced the wound area in skin tissue on day 8. Histological analysis showed that both HN and f-HN accelerated wound healing by inducing neovascularization and increasing collagen production to improve re-epithelialization. In vitro assay results in cell migration and expression of the angiogenesis-related genes, were promoted by f-HN. Furthermore, both MDPs enhanced the phosphorylation of signal transducer and activator of transcription 3 (STAT3) protein expression. Of note, f-HN promoted cell migration through the STAT3 signaling pathway, and it was suppressed by BP-1-102, a STAT3 inhibitor. Together, these results identify an underlying regulatory mechanism of MDPs in mouse skin wound healing via the STAT3 signaling pathway and proposed MDPs as a potential therapeutic for tissue repair. This study was supported by the National Research Foundation of Korea (NRF-2017R1A5A2015369 & NRF-2022R1A2C2011079).

Keywords: Mitochondrial-derived peptides, Humanin, Formylated Humanin, Skin wound healing, Angiogenesis, STAT3 signaling

P-07-002

The Role of JAK3 in Skin Wound Healing

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The skin is a tissue that protects the body from harmful substances in the external environment. If the skin is injured, the skin initiates a wound healing process to heal wounds quickly. The wound healing process consists of the Inflammatory Phase, Proliferative Phase and remodeling phase. It is known that fibroblast cells are involved in skin contraction during this phase and fibroblast cells secrete extracellular matrix such as collagen. Taken together, Not only the immune system, but also contraction is important for recovering wounds quickly.

JAK is known as a non-receptor tyrosine kinase that mediated in cytokines and various growth factor signaling through the JAK-STAT pathway. Among the proteins involved in the JAK-STAT pathway, JAK3 is well known to be plays a particularly important role in the activation of the immune system. Furthermore, JAK has been found to affect cell growth, survival, development, differentiation, and migration of neocortical neuroblasts. In our previous study,

In order to study the function of JAK3 on the skin, I made wounds to the mouse back skins and a JAK3 inhibitor was administered. As a result, skin contraction was significantly decreased by JAK3 inhibition. In addition, the thickness of the epidermis of the wound bed, the thickness of the healed muscle layer, and area of sebocytes were also decreased through H&E staining, trichrome staining, and immunohistochemistry. These results were caused by fibroblast cells. Therefore, primary fibroblast cell culture was performed to observe changes in fibroblast cells caused by JAK3 inhibition. As a result, the proliferation of fibroblast cells was reduced and the morphology was sharply changed.

Keywords: JAK3, Skin, Fibroblast, Sebaceous gland

P-07-003

Hyperbaric oxygen therapy promotes diabetic wound healing via AKT and ERK signaling pathway

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Diabetes mellitus is a chronic disease that greatly impacts the quality of life and survival expectancy of patients. A chronic diabetic wound is one of the severe complications of diabetes reducing the quality of life. Although hyperbaric oxygen therapy (HBOT) is known as an effective therapy for diabetic foot ulcers, diabetic wound healing still has many challenges in uncovering the most effective HBOT condition and the mechanism for the wound healing process. Here we examined the effect of HBOT in 3 different groups (2, 2.5, and 3 ATM) and non-HBOT as a control for treating in vivo diabetic skin wounds. After wound production in twelve-week-old diabetic mice, the mice are treated with HBOT for 2 hrs, 3 days per week for 2 weeks. Histological analysis of 2.5 ATM condition treatment showed the smallest wound area, thickened epidermal and dermal thickness, elevated collagen composition, and enhanced re-epithelization. Moreover, ERK1/2 and NRF2 signaling were upregulated in skin tissues and fibroblast cells under 2.5 ATM treated conditions. Additionally, HBOT suppressed AKT phosphorylation to promote angiogenesis and decrease inflammation. Together, the results suggest that 2.5 ATM is the most effective condition of HBOT for diabetic wound healing processes, and that provides the clue for therapeutic intervention of wound healing.

Keywords: HBOT, Skin, Wound healing, Diabetic mice

P-07-004

Bitter taste, a possible new function.

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Taste is closely related to intake of food. Taste perception is also influenced by type of food ingested, and nutrition and health status. Bitter taste plays an important role in the survival of human and animals to avoid probable toxic and harmful substances. 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 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. BPs were measured by non-invasive technique on tail. FBGs were monitored by Accu-Chek® Instant. Wild type C57BL/6 mice or tas2r108 knock-out mice from 8 to 78-week-old were used.

Tas2r108 knock-out does not elicit much change in expression of 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 results suggest that at least by 18 months tas2r108 KO would elicit change in metabolism or feeding behavior. However, more continuous research is needed to confirm the exact physiological role of bitter taste.

Acknowledgement: The work is supported by Basic Science Research Program through the National Research Foundation in Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (2020R1F1A1049633).

Keywords: Tas2r108, Taste gene expression, Bitter taste, Blood pressure, Metabolism, Feeding behaviour

P-08-001

Effects of trehalose, an autophagy enhancer on implant surface and inflamed and infected bone.

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Autophagy has known to be involved in regulating inflammation of various tissue and odontogenic differentiation. Bone inflammation and infection is one of major causes in dental implant failure but their prevention has been poorly established. This study is aimed to investigate whether autophagy enhancer is available for therapeutic reagent for reducing bone inflammation and up-regulating osteoblast differentiation.

LPS or P. gingivalis inhibited osteogenic differentiation in MC3T3-E1 cell by up-regulating release of inflammation cytokine produced from macrophage. Trehalose, an autophagy enhancer, rescued LPS or P. gingivalis-induced impaired osteogenic differentiation from MC3T3-E1 cell co-cultured with Raw 264.7 cell through reducing inflammatory cytokine, IL-6, IL-1b. Also, implants coated with trehalose showed an increment of new bone formation and a decrement of inflammation in the rabbit calvarium under LPS or P. gingivalis treatment.

These results suggest that autophagy reduce bone inflammation through inhibiting inflammatory cytokine release from macrophage as well as autophagy enhancer coated implant may be available for inflamed and infection bone.

Keywords: Trehalose, Autophagy, Inflammation, Osteoblast differentiation

P-08-002

In vivo administration of Gas6 inhibits epithelial-mesenchymal transition and enhances PGE2 and PGD2 in alveolar type II epithelial cells following bleomycin treatment

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The epithelial-mesenchymal transition (EMT) process is a major event in idiopathic pulmonary fibrosis (IPF) pathogenesis. Here, we investigated the in vivo role of growth arrest-specific protein 6 (Gas6) in bleomycin-induced EMT process. Administration of Gas6 inhibited EMT process in isolated alveolar epithelial type II cells (ATII) at 14 days post-bleomycin treatment into mice, based on morphologic cellular alteration, changes in mRNA and protein expression profiles of EMT markers, including loss of E-cadherin, synthesis of N-cadherin and α -smooth muscle actin (α -SMA), and induction of EMT-activating transcription factors, such as Snai1, Zeb1, and Twist1. Gas6 administration induced phosphorylation of Axl and Mer receptor tyrosine kinases, and cyclooxygenase-2 (COX-2) expression at mRNA levels and prostaglandin E2 (PGE2) and PGD2 levels in the supernatants of cultured primary ATII following bleomycin treatment. Additionally, administration of Gas6 reduced mRNA and protein levels of α -SMA, fibronectin and type 1 collagen α 1 in lung tissue following bleomycin treatment. Our data suggest Gas6 signaling events play a potential role in resistance to EMT process via COX-2-derived PGE2 and PGD2 production.

Keywords: Gas6, Bleomycin, EMT, PGE2, PGD2, Lung fibrosis

P-08-003

CRIF1 upregulated homocysteine production by suppressing DHFR expression in vascular endothelial cellsMinsoo Kim¹, Shuyu Piao¹, Seonhee Kim¹, GiangHuong Vu¹, Ikjun Lee^{1,2}, Cuk-seong Kim^{1,2}¹Department of physiology Department of Medical Science, Chungnam National University, ²Department of physiology Brain Korea ²¹ FOUR Project for Medical Science, Chungnam National University

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

P-08-004

Optimization and characterization of exosomes from mouse brain: evaluation of it for pathogenic role in delayed onset brain injuryJong Hun An^{1,2,3}, Jiebo Zhu^{1,2,3}, Min Joung Lee^{1,2,3}, Jun Young Heo^{1,2,3}¹Department of Biochemistry Chungnam National University School of Medicine, Daejeon, South Korea, ²Department of Medical Science Chungnam National University School of Medicine, Daejeon, South Korea, ³Infection Control Convergence Research Center Chungnam National University School of Medicine, Daejeon, South Korea

Secreted exosomes which contained intracellular proteins, miRNAs, mRNAs act as messenger to inform the adjacent cells. To date, through analysis of brain exosomes, several biomarkers on brain injury have been revealed and contribute the early diagnosis of it. Despite the efforts on exosome analysis in brain, there is currently limited studies on pathogenic role of exosome during blood brain barrier (BBB) disruption after brain injury. Previously, we reported modified method for isolation of microvessel (MV) from mouse BBB. To begin with this study, we optimized the isolation of exosomes from MV by comparing the ultracentrifugation (UC) and ultrafiltration (UF). First, we identified the exosomes on MV by co-immunostaining with CD63 (represent for exosome) and CD31 (represent for endothelial cell). Next, we assessed size and number of particles with nanoparticle tracking analysis (NTA) after isolation of exosome from mouse brain. Comparing with UC and UF, we choose exosome isolation protocol based on high yield and purity grade. With the three independent experiment of exosome isolation from MV in brain, isolation by using UC acquired the higher purity of exosomes than UF. According to recent reports, intracerebral hemorrhage patients has been suffering for remaining symptoms after complete remission which called delayed onset injury (DOI). To identify the DOI, we generated the ICH model by collagenase injection to the striatum and evaluated the neuroinflammation in thalamic area after 14 days. Interestingly, we found that increase of CD11b and CD63 positive cells in thalamic area of ICH mice com-

paring with vehicle injected mice which represent microglia activation and exosomes respectively. Moreover, reinjection of the isolated MV exosomes from ICH mice to the thalamic area 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.

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Keywords: Exosome, BBB, Micro vessel, Exosome isolation, Delayed onset brain injury

P-08-005

Ulinastatin Attenuates Vascular Damage in IDH2-Deficient Endothelial Cells via TGF- β /MMP7/SDS2 signaling pathwayGianghuong Vu^{1,2}, Sujeong Choi¹, Shuyu Piao¹, Seonhee Kim¹, Minsoo Kim^{1,2}, Byeonghwa Jeon^{1,2}, Cukseong Kim^{1,2}¹Department of physiology Department of Physiology & Medical Science, College of Medicine, Chungnam National University, ²Department of physiology Brain Korea 21 FOUR project for medical science, Chungnam national University

Mitochondrial dysfunction contributes to several acute and chronic inflammatory diseases. We previously showed that a deficiency in isocitrate dehydrogenase 2 (IDH2) in endothelial cells led to mitochondrial dysfunction, and endothelial inflammation. Syndecan-2 (SDC2) is a glycoprotein from Syndecan family that is highly expressed in endothelial cells, especially during endothelial inflammation. Therefore, we investigated the association between inhibition IDH2 and SDS2 expression in vascular endothelial cells. We observed that SDS2 expression was increased in IDH2-deficient human umbilical vein endothelial cells (HUVECs). Matrix metalloproteinase 7 (MMP7) impacted on SDC2 expression via TGF- β pathway. Furthermore, the changes caused by IDH2 deficiency was reversed by Ulinastatin (UTI) treatment. Similarly, administration of UTI decreased SDS2, MMP7, and TGF- β expression in the aorta of IDH2 knockout mice. These data showed that UTI had protective effect on the vascular damage caused by downregulation of IDH2 via the TGF- β /MMP7 signaling pathway in endothelial cells.

Keywords: Ulinastatin, IDH2, SDS2, Endothelial Cells, Vascular Damage

P-08-006

Protective effect of myricetin in RINm5F β -cells under exposure to interleukin-1 β

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Myricetin, a naturally occurring flavonoid, has the beneficial effects of preserving pancreatic β -cell mass and function. Especially the pro-inflammatory cytokine, interleukin-1 β (IL-1 β) is crucial as a mediator leading to β -cell destruction. The aim of the present study was the evaluation of possible protective effects of myricetin against IL-1 β -induced cytotoxicity in pancreatic RINm5F β -cells. The results showed that myricetin increased cell viability and decreased cell apoptosis induced by IL-1 β via regulation of expression of apoptotic signaling molecules. Moreover, myricetin prevented β -cell oxidative stress in response to IL-1 β by decreased concentration of reactive oxygen species (ROS) and/or nitric oxide (NO). Specifically, myricetin inhibited the iNOS-mediated NO synthesis dose-dependently, and reduced the levels of iNOS mRNA and its protein expression in IL-1 β -treated RINm5F β -cells. Myricetin also partially inhibited NF- κ B activation for iNOS induction, I κ B phosphorylation and NF- κ B p65 nuclear translocation, but not NF- κ B DNA binding activity. Myricetin also decreased the expression of iNOS mRNA through reduction of mRNA stabilization. In addition, myricetin reduced the stability of iNOS protein in cycloheximide-treated. All of these

findings showed the direct cytoprotective effect of myricetin on pancreatic RINm5F β -cells.

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Keywords: Myricetin, INOS transcription, mRNA stability, Protein stability, RINm5F β -cells

P-08-007

Alleviation of inflammatory parameters by fermented and aged mountain cultivated ginseng sprouts and its main component, compound K, in acute lung injury and asthma models

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Acute lung injury (ALI) and asthma are inflammatory respiratory diseases. Ginseng has long been used as a health functional food due to its anti-inflammatory and immune-modulating effects. By adding antioxidant activity to these properties, the value of ginseng as a nutraceutical can be increased. This study investigated the effects of fermented and aged mountain-cultivated ginseng sprout (FAMCGS) extracts and its main component, compound K (CK), in ALI and asthma models. The FAMCGS had higher antioxidant activity than MCGS through aging and fermentation processes. Inflammatory signals (the number of inflammatory cells, production of pro-inflammatory cytokines, mucus secretion, macrophage infiltration, and NF- κ B activation) increased in lung tissue and bronchoalveolar lavage fluid (BALF) obtained from LPS-challenged ALI mice were significantly decreased in the combined group of FAMCGS or CK and LPS. Ovalbumin (OVA)-induced asthmatic inflammation was also reduced in OVA+FAMCGS and OVA+CK groups. In addition, the FAMCGS and CK combination markedly lowered the ROS production induced in each model. FAMCGS and CK showed therapeutic effects in LPS-induced ALI and OVA-induced asthma models. Primary macrophages and a macrophage cell line were employed to determine the therapeutic mechanism of FAMCGS and CK. LPS- or IL4-induced macrophage activation was inhibited by FAMCGS and CK. LPS-induced increases in M1 markers (iNOS, IL-1 β , IL-6, and TNF- α) were decreased by pretreatment with FAMCGS or CK. IL4-induced increases in M2 markers (Arg-1, CD206, and Ym-1) were down-regulated by FAMCGS or CK pretreatment. In addition, FAMCGS and CK inhibited macrophages' ROS induced by LPS and IL-4. These results showed that FAMCGS and CK had therapeutic effects in LPS-induced ALI and asthma mouse models by inhibiting inflammatory and oxidative parameters through the downregulation of macrophage polarization. Our results suggest that FAMCGS and CK can be potential therapeutic agents for ALI and asthma respiratory diseases.

Keywords: Acute lung injury, Asthma, Compound K, FAMCGS, Inflammation

P-08-008

Elevated Plasma Apurinic/Apyrimidinic Endonuclease 1/Redox Effector Factor-1 Levels in Refractory Kawasaki Disease

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Kawasaki disease is an acute febrile systemic vasculitis syndrome of unknown etiology that occurs mainly in children under 5 years of age. There is increasing evidence that apurinic/aprimidinic endonuclease 1/redox factor-1 (APE1/Ref-1) regulates inflammatory responses. In this study, we investigated the association between APE1/Ref-1 and KD. Three groups, including 32 patients with KD (KD group), 33 patients with fever (Fever group), and 19 healthy individuals (Healthy group), were prospectively analyzed. APE1/Ref-1 levels were measured, and the clinical characteristics of KD were evaluated. The mean age of all patients was 2.7 ± 1.8 years, but the Healthy group participants were older than the other participants. Fever duration was longer in the KD group than in the fever group. APE1/Ref-1 levels were significantly higher in the KD group ($p = 0.004$) than in the other two groups, but there was no difference between the healthy and fever groups. APE1/Ref-1 levels did not differ according to fever duration or coronary arterial lesion but were higher in refractory KD cases than in non-refractory cases. APE1/Ref-1 levels were significantly higher during the acute phase of KD. We propose that APE1/Ref-1 could be a beneficial biological marker for the diagnosis and prognosis of KD, especially in refractory KD.

Keywords: Apurinic/aprimidinic endonuclease-1/redox factor-1, Mucocutaneous lymph node syndrome, Vasculitis

P-09-001

In vivo injection of apoptotic cancer cells inhibits CAF activation and lung metastasis via Notch1-WISP-1 signaling

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Here, we used syngeneic (129/Sv) immunocompetent mice to investigate the in vivo response of cancer-associated fibroblasts (CAFs) to apoptotic 344 SQ cells (ApoSQ) injection after subcutaneous injection of 344SQ cells. mRNA expression of traditional CAF activation markers in isolated Thy1⁺ CAFs was reduced after ApoSQ injection. However, mRNA expression of *Notch1* and *Notch* downstream target genes was enhanced after ApoSQ injection. To confirm the inhibitory role of Notch1-WISP-1 signaling in CAF activation and tumor progression *in vivo*, administration of the Notch1 selective inhibitor LY3039478 did not alter body weight, primary tumor weight or volume compared with control and ApoSQ groups. However, LY3039478 reversed the ApoSQ-induced reduction in tumor nodule number on the lung surface and the rate of metastasis. LY3039478 also reversed the ApoSQ-induced reduction in mRNA expression of CAF markers and enhancement in mRNA expression of Notch1 and Notch downstream target genes in Thy1⁺ CAFs. Taken together, these results demonstrate that single injection of ApoSQ inhibits the activation of CAFs, but enhances Notch1-WISP-1 signaling in CAFs. In addition, Notch1-WISP-1 signaling in CAFs targeted by ApoSQ injection may play a critical role for anti-CAF activation and anti-metastatic nature.

Acknowledgement: This work was supported by the National Research Foundation of Korea grants funded by the Korean government (MSIT) (2020R1A5A2019210 and 2020R1A2B5B02001686).

Keywords: Apoptotic lung cancer cells, Cancer-associated fibroblasts, Notch1 inhibitor LY3039478, WISP-1, Metastasis

P-09-002

SUV39H1-driven NFATc1 methylation is essential for the c-Cbl-mediated degradation of NFATc1 in an osteoclast lineage

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Bone undergoes continuous remodeling by tightly-coordinated actions of bone-resorbing osteoclasts and bone-forming osteoblasts. Recent studies document that the dysregulation of histone methylation profiles is associated with the progression of osteoclastogenesis. However, the specific epigenetic modifiers are incompletely understood. In this study, we demonstrated an inhibitory role of a variegation 3-9 homolog 1 (SUV39H1) in osteoclast formation. Public datasets find that mRNA levels of several methyltransferases are gradually decreased during osteoclastogenesis. Among them, inhibition of SUV39H1 leads to accelerated osteoclast differentiation with the induction of several osteoclastogenic genes both in vivo and in vitro. In this regard, SUV39H1 directly binds and methylates a nuclear factor of activated T cells 1 (NFATc1) protein at lysine 267 of the RD motif. Importantly, SUV39H1 enhances the crosstalk between post-translational modification involving NFATc1 methylation and degradation. Inhibition of SUV39H1-mediated NFATc1 methylation significantly decreases c-Cbl-dependent NFATc1 ubiquitination and degradation. Finally, SUV39H1 methylates lysine 9 of histone 3 at osteoclastogenic gene promoters, thereby repressing NFATc1 transcriptional activity. Taken together, our findings reveal that SUV39H1 plays both enzymatic and epigenetic roles in its action as an intrinsic suppressor of osteoclast differentiation.

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Keywords: NFATc1, SUV39H1, Osteoclastogenesis, Methylation

P-09-003

Tumor-Treating Fields (TTFields), an Anti-Cancer Therapeutic Modality, Induces Cell Death in Liver Cancer Cell

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Despite enhanced survival outcomes across many tumor types, the prognosis remains bleak for certain organ tumors including hepatoma and glioblastoma tumor. Always in these tumors, the regulation achieved by time-limited interventions such as typical chemotherapy, radiation therapy and surgical resection is short-lived. A current form of anti-tumor therapy called tumor-treating fields (TTFields) has been shown, in combination with chemotherapy or either by itself, to have anti-tumor effects that translate to enhanced survival outcomes in tumor patients. A novel therapy, now usually referred to as TTFields, was approved by the FDA in 2011 for use in glioblastoma (GBM). The FDA approved 200 KHz TTFields for GBM after phase III clinical trial, which TTFields in combination with adjuvant chemotherapy prolonged median overall survival by 4.9 months compared with

the adjuvant chemotherapy alone. Even though the (pre-) clinical date is promising, the mechanisms of TTFields is not only completely explained. Many research is underway to better understand how and why TTFields is able to selectively kill the tumors and block their proliferation. The TTFields is a recent tumor treatment modality that uses alternating, low intensity (1-4 V/cm) electric fields at median frequencies (100-300 KHz) to disrupt tumor cell proliferation. The present study evaluated the combination of TTFields and barium titanate nanoparticle (BTNP) on liver cancer cell derived from C3H mouse. We exposed mouse HCA-1 cells with TTFields (150 KHz for 72 hr) and BTNPs and detected cell death using immunofluorescence staining and crystal violet staining. High-performance holotomography microscope (HT-X1) further showed that the number of nanoparticles entering the HCA-1 cells and increasing the cell death in compared with untreated TTFields and BTNPs. The findings of the present study provide meaningful evidence for the further advancement of combination with the TTFields and BTNPs in the HCA-1 liver cancer cells derived from C3H mouse.

Acknowledgement: This study was supported by the Internal Research Program of Electronics and Telecommunications Research Institute.

Keywords: Tumor-treating fields, Liver cancer, Therapeutic effects

P-09-004

Apoptotic cancer cells stimulate WISP-1 secretion from cancer-associated fibroblasts (CAFs) to inhibit migration and invasion of lung cancer cells and CAFs

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We investigated whether paracrine/autocrine factors secreted from cancer-associated fibroblasts (CAFs) after exposure to apoptotic cancer cells are critical for inhibiting migration and invasion of lung cancer cells and CAFs. We performed cytokine array measuring up to 111 cytokines. Among the cytokines, Wnt-induced signaling protein 1 (WISP-1) and leukemia inhibitory factor (LIF) were most highly increased. Ultimately, we determined which cytokine inhibits migration and invasion using specific siRNAs of WISP-1 and LIF. WISP-1 knockdown reduced WISP-1 secretion from CAFs and reversed the anti-migration and -invasion effects of condition medium from CAFs exposed to apoptotic 344SQ cells (ApoSQ-CAF CM) and ApoSQ on 344SQ cells and CAFs, respectively. By contrast, LIF knockdown which reduced ApoSQ-induced LIF secretion, but not WISP-1 secretion, from CAFs, did not affect the migration and invasion of 344SQ cells or CAFs. Additionally, treatment with ApoSQ-CAF CM after neutralization with anti-WISP-1 antibodies reversed the anti-migration and -invasion effects on 344SQ cells. Notably, WISP-1 overexpression in CAFs enhanced WISP-1 secretion with or without ApoSQ. CM from WISP-1-overexpressing CAFs cultured with ApoSQ further inhibited the migration and invasion of 344SQ cells compared with CM from mock-transfected CAFs cultured with ApoSQ. Collectively, these results suggest that WISP-1 secretion from CAFs exposed to ApoSQ mediates the anti-migration and -invasion effects in lung cancer cells and CAFs.

Keywords: Cancer-associated fibroblasts, Apoptotic lung cancer cells, Migration, Invasion, WISP-1, LIF

P-09-005

Enhancement of the Notch ligand Dll1 expression in UV-irradiated apoptotic cancer cells activates Notch1 signaling in cancer-associated fibroblasts (CAFs)

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Most Notch signaling is initiated by interactions between the cell-surface

receptor Notch and a cell-bound ligand, such as Dll1, 3, or 4 or Jagged-like (Jag) 1 or 2. To investigate the cell-surface interaction required for activation of Notch1 signaling in cancer-associated fibroblasts (CAFs), we first examined expression of Notch ligands on the apoptotic cell surface using flow cytometry. Dll1 expression was enhanced in UV-irradiated apoptotic cancer cells, including 344SQ, A549, and HCT116 cells. However, the expression of other ligands did not change or was decreased. Western blot confirmed enhanced Dll1 expression only in apoptotic cancer cell lysates. The neutralization of Dll1 with anti-Dll1 antibodies on the surface of apoptotic 344SQ cells (ApoSQ) diminished ApoSQ-induced Notch1 signaling and Wnt-induced signaling protein 1 (WISP-1) secretion by CAFs. Similarly, knockdown of Dll1 in 344SQ cells by transfection with specific siRNA before apoptosis induction by UV-irradiation inhibited Notch1 activation and WISP-1 secretion by ApoSQ cells. These findings suggest that Notch1 signaling in CAFs is initiated by an interaction with adjacent apoptotic cancer cells expressing Dll1.

Acknowledgement: This work was supported by the National Research Foundation of Korea grants funded by the Korean government (MSIT) (2020R1A5A2019210 and 2020R1A2B5B02001686).

Keywords: Cancer-associated fibroblasts, Apoptotic cancer cells, Notch ligand Dll1, WISP-1

P-09-006

Neddylation blockade accelerates cancer cell migration under the condition of insulin resistance

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Background: Malfunction in insulin such as hyperinsulinemia and insulin resistance, is not only linked to obesity and diabetes but also to increased risk of different types of cancer. It has been proposed that hyperinsulinemia is associated with tumor aggressiveness, however the mechanistic details are yet to be elucidated.

Methods: We utilized different kinds of cancer cell lines by inducing insulin resistance under the condition of neddylation blockade. Cell migration were assessed through wound healing and transwell assays. We investigated the molecular events driving the cell migration using quantitative real time-PCR and immunoblotting.

Results: We recently reported the role of neddylation in cancer cell migration. Here, we found that under the condition of insulin resistance, inhibition of neddylation further induced migration in different cancer cell lines through activation of insulin receptor substrate IRS1 and the downstream PI3K/Akt pathway.

Conclusions: Altogether, the data suggests the possible role of neddylation in cancer cell migration under the condition of insulin resistance. It also implies the possible risk of applying neddylation inhibitors as anticancer regimens for patients exposed to obesity or diabetes, which should be carefully evaluated before clinical application.

Keywords: Neddylation blockade, Hyperinsulinemia, Cancer, Metastasis

P-09-007

Atractylodes macrocephala Koidz induces apoptosis in human gastric cancer cells through Activation of the ROS and MAPK Signaling Pathway

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Atractylodes macrocephala Koidz (AMK) is a traditional medicine used to treat various diseases, including gastrointestinal diseases, cancer, osteoporosis, Alzheimer's disease, and others. However, the anti-gastric cancer and

apoptotic effects of the ethanol extract of the AMK remain unknown. DNA content analysis indicated that AMK increased the sub G1 population of AGS cells. In addition, levels of proapoptotic cascade components, including caspase-3, caspase-9 and poly ADP ribose polymerase, were augmented by AMK treatment. Mitochondrial membrane potential was reduced, and the ratio of Bcl-2 associated X protein (Bax)/ B cell lymphoma-2 (Bcl-2) were increased, also intracellular reactive oxygen species (ROS) production was also increased by AMK treatment. The AMK induced cytotoxic effects and ROS production could be attenuated by N-acetyl-cysteine (NAC), an ROS scavenger. Taken together, these results indicate that AMK is a potent apoptotic herbal medicine, which exerts its effects via the ROS mediated mitochondrial pathway. These findings suggest that AMK induced apoptosis in AGS cells and thus might serve as a novel anticancer agent to promote apoptosis of gastric cancer cells.

Keywords: Atractylodes macrocephala Koidz (AMK), Gastric cancer, Anti cancer, Apoptosis, ROS

P-09-008

High diagnostic and therapeutic performance of exosomal miR-34 family for brain metastasis in lung cancer

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Exosomal microRNAs (miRNAs) have been identified as a key factor mediating communication between cancer and stromal cells in the tumor micro-environment. However, whether cancer cell-derived exosomal miRNAs are involved in the development of metastatic brain cancer is largely unknown. Brain metastasis occurs in 30-50% of the cancer patients, and lung cancer is the most common causative primary cancer. The penetration of the blood-brain barrier by circulating tumor cells is a key event in brain metastasis, and recent studies have shown that exosomes play an important role in this process. The purpose of this study was to identify the diagnostic and prognostic markers of brain metastasis in lung cancer by analyzing exosomal miRNAs in a syngeneic lung cancer mouse model. We performed exosome RNA sequencing to compare the levels of miRNA/mRNA whose expression changed in case of brain metastasis. We found that the levels of miR-34 family members were significantly reduced in the serum exosome samples derived from lung cancer with brain metastasis relative to samples without brain metastasis. Further, we found that the target genes of miR-34 family, which are involved in the formation of a favorable premetastatic niche, were highly expressed at the mRNA and protein levels in brain metastases than in normal or non-metastatic samples. Our results show that the miR-34 family can serve as a novel diagnostic and prognostic marker, and may serve as a therapeutic target for brain metastasis in lung cancer.

Keywords: Lung cancer, Brain metastasis, Exosome, MiR-34 family

P-09-009

Effects of N-acetyl cysteine and buthionine sulfoximine in propyl gallate-treated lung cancer cells: cell death, reactive oxygen species, and glutathione

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Propyl gallate (3,4,5-trihydroxybenzoic acid propyl ester; PG) has an anti-growth effect in lung cancer. The present study investigated the effects of N-acetyl cysteine (NAC) and L-buthionine sulfoximine (BSO) on cell growth

and death in PG-treated Calu-6 and A549 lung cancer cell lines regarding reactive oxygen species (ROS) and glutathione (GSH) levels. Treatment with 800 μ M PG-induced growth inhibition and cell death in Calu-6 and A549 cells at 24 h, accompanied by a loss in mitochondrial membrane potential (MMP; $\Delta\Psi_m$). NAC treatment (2 mM) attenuated this inhibition in Calu-6 cells but not A549 cells and significantly increased the numbers of sub-G1 or annexin V-positive cells in PG-treated A549 cells, but not Calu-6 cells. BSO treatment (10 μ M) promoted cell death and MMP loss resulting from PG treatment in both lung cancer cell lines. Moreover, PG increased ROS levels and caused GSH depletion in both cancer cell lines. NAC decreased ROS levels in PG-treated Calu-6 cells but increased $O_2^{\cdot-}$ levels in both PG-treated lung cancer cells. BSO increased ROS levels in PG-treated A549 cells and increased $O_2^{\cdot-}$ levels in PG-treated Calu-6 cells. In addition, NAC strongly promoted GSH depletion in both PG-treated lung cancer cells. Moreover, BSO increased GSH depletion in PG-treated cells but not A549 cells. In conclusion, NAC promoted PG-induced A549 cell death by increasing ROS levels and depleting GSH. BSO increased PG-induced Calu-6 cell death by increasing $O_2^{\cdot-}$ levels and depleting GSH.

Acknowledgement: 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 (2019R111A2A01041209).

Keywords: Lung cancer, Propyl gallate, Cell death, N-acetyl cysteine, L-buthionine sulfoximine

P-09-010

Tempol inhibits the growth of lung cancer and normal cells through apoptosis accompanied by increased $O_2^{\cdot-}$ levels and glutathione depletion

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Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl) is a stable, cell-permeable redox-cycling nitroxide water-soluble superoxide dismutase (SOD) mimetic agent. However, little is known about its cytotoxic effects on lung-related cells. Thus, the present study investigated the effects of Tempol on cell growth and death as well as changes in reactive oxygen species (ROS) and glutathione (GSH) levels in Calu-6 and A549 lung cancer cells, normal lung WI-38 VA-13 cells, and primary pulmonary fibroblast cells. Results showed that Tempol (0.5 ~ 4 mM) dose-dependently inhibited the growth of lung cancer and normal cells with an IC_{50} of approximately 1 ~ 2 mM at 48 h. Tempol induced apoptosis in lung cells with loss of mitochondrial membrane potential (MMP; $\Delta\Psi_m$) and activation of caspase-3. There was no significant difference in susceptibility to Tempol between lung cancer and normal cells. Z-VAD, a pan-caspase inhibitor, significantly decreased the number of annexin V-positive cells in Tempol-treated Calu-6, A549, and WI-38 VA-13 cells. A 2 mM concentration of Tempol increased ROS levels, including $O_2^{\cdot-}$ in A549 and WI-38 VA-13 cells after 48 h, and specifically increased $O_2^{\cdot-}$ levels in Calu-6 cells. In addition, Tempol increased the number of GSH-depleted cells in Calu-6, A549, and WI-38 VA-13 cells at 48 h. Z-VAD partially downregulated $O_2^{\cdot-}$ levels and GSH depletion in Tempol-treated these cells. In conclusion, treatment with Tempol inhibited the growth of both lung cancer and normal cells via apoptosis and/or necrosis, which was correlated with increased $O_2^{\cdot-}$ levels and GSH depletion.

Acknowledgement: 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 (2019R111A2A01041209).

Keywords: Tempol, Lung cancer cells, Human pulmonary fibroblast, Cell death, Mitochondrial membrane potential, Reactive oxygen species, Glutathione

P-09-011

Identification of the role of SIRT6 as a tumor suppressor in liver cancer

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The sirtuin 6 (SIRT6) gene belongs to the family of NAD-dependent class III sirtuins and it has been known that sirtuin 6 plays an important role in many areas including cancer. The purpose of this study is to elucidate the role and function of sirt6 in liver cancer. SIRT6 was highly expressed in human liver cancer. Silencing SIRT6 increased liver cancer cell viability while overexpressing SIRT6 suppressed it. Overexpression of SIRT6 increased expression of cleaved-PARP and cleaved-caspase9 and decreased the PARP, caspase9, and caspase3. Knockdown of SIRT6 increased the number and size of colonies. In addition, overexpression of SIRT6 significantly inhibited the invasion and metastasis of liver cancer cells whereas silencing of SIRT6 increased the invasion and metastasis abilities of liver cancer cells in a time dependent manner. Silencing SIRT6 in liver cancer cells increased the levels of vimentin, UPA, and MMP9 protein, while overexpression of SIRT6 inhibited them. Autophagy marker LC3-II/LC3-I and P62 was changed after SIRT6 increased or decreased in liver cancer cells indicated sirt6 may stimulate autophagy in liver cancer cells. In vitro, knockdown of SIRT6 significantly promoted the tumor growth. As a result, SIRT6 may play a role in tumor suppression in HCC cells, suggesting that it may be a protumorigenic factor in the development of liver cancer.

Keywords: SIRT6, Hepatocellular carcinoma cells, Metastasis, Cell proliferation, Autophagy

P-09-012

A role of Hematopoietic- and neurologic-expressed sequence 1 in ER-stress and autophagy in Hepatocellular carcinoma cells

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Since liver cancer is an aggressive illness with a high incidence and fatality rate, there is an urgent need for new molecular targets and efficient treatments. The purpose of the current study is to investigate HN1's underlying mechanism in HCC. HN1 knockdown produced G1 cell cycle arrest and increased cell death. Additionally, HN1 knockdown increased autophagy but HN1 overexpression decreased it. Although chloroquine-induced autophagy decrease was restored, HN1 under starving conditions or treatment with Torin 1 further increased autophagy. The mTOR/AKT signaling pathway and the Bcl-2/Bax signaling pathway were both downregulated by HN1 knockdown. Under starvation conditions or Torin 1 therapy, HN1 knockdown boosted the nuclear-translocation of transcription factor EB (TFEB), an important transcriptional regulator of lysosome formation and autophagy. All of these findings point to HN1 knockdown inducing autophagy in HCC. Furthermore, knockdown of HN1 induced ER stress and overexpression of HN1 counteract part of tunicamycin induced-ER stress. Our findings imply that HN1 controls the ER stress, autophagy, and cell death of hepatocellular carcinoma cells.

Keywords: HN1, ER stress, Autophagy, TFEB, Hepatocellular carcinoma

P-09-013

Enhanced efficacy of 5-fluorouracil combined with histone deacetylase inhibitor panobinostat against Gastric Cancer

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As a histone deacetylase (HDAC) inhibitor, panobinostat inhibits cancer cell growth, metastasis, and tumorigenesis in various cancers. A traditional chemotherapy reagent, 5-fluorouracil (5-Fu), has limited its curative effects in gastric cancer due to resistance to chemotherapy and side effects. The present study aimed to investigate whether panobinostat promotes the anti-tumor effect of 5-Fu. Combination treatment with Panobinostat and 5-Fu inhibited cell proliferation and cell growth more effectively than either treatment alone by inducing more significant apoptosis, as indicated by increased protein levels of cleaved-PARP and cleaved caspase-9. Similar to the cell growth suppressive effect, the combination of panobinostat and 5-Fu significantly inhibited cell migration by targeting E-cadherin, MMP-9, and Vimentin in gastric cancer cells. Thus, panobinostat effectively potentiates the anti-tumor efficacy of 5-Fu since combination treatment shows significantly more anti-tumor potential than 5-Fu alone. Patients with gastric cancer may benefit from a combination treatment with panobinostat and 5-Fu.

Keywords: 5-fluorouracil, Panobinostat, Apoptosis, Gastric Cancer

P-09-014

Effects of rapamycin and hydroxychloroquine in auranofin-treated lung cancer cells: cell death, reactive oxygen species, 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 μ M and 6 μ M at 24 h, respectively. Auranofin induced apoptosis and necrosis in these cell lines, which were accompanied by the loss of mitochondrial membrane potential (MMP; $\Delta\Psi$ m). 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-1 and P62 in auranofin-treated Calu-6 and A549 cells. Treatment with 20 μ M HQ increased cell growth inhibition, MMP loss, ROS levels and GSH depletion in auranofin-treated Calu-6 and A549 cells. However, treatment with 100 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.

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Keywords: Auranofin, Lung cancer cells, Hydroxychloroquine, Rapamycin, Reactive oxygen species, Glutathione

P-09-015

The molecular mechanism of TMEM16E-mediated plasma membrane repair (PMR) system

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Transmembrane protein 16E (TMEM16E), a Ca²⁺-activated phospholipid scramblase, regulates diverse biological functions including apoptosis and blood coagulation by altering the physical properties of the cell membrane. Recent studies reported that TMEM16E, a Ca²⁺-activated phospholipid scramblase promotes plasma membrane repair (PMR) system after injury pore-forming toxins by reducing membrane tension and facilitating release of extracellular vesicles containing damaged membranes. Here, we found that TMEM16E protected the cells by inducing the macropinocytosis of phospholipid-scrambled plasma membrane. In normal condition intracellular Ca²⁺ 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. In our results, however, in cells expressing the TMEM16E, the membrane-targeted AV-molecules were rapidly internalized to the cells in pH 7.4. 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, the scramblase activity of TMEM16E was dramatically reduced and the internalization of AV-molecules was enhanced at pH 5.5 solution. Additionally, the internalization of AV-molecules contributed to maintaining the cell morphology in normal. Finally, we confirmed that intracellular Ca²⁺ level was highly modulated by extracellular proton levels and lower pH speeded up the Ca²⁺ clearance pathways probably by activating the cation/Ca²⁺ exchangers, demonstrating that the extracellular acidification appearing in cells expressing TMEM16E can further enhance cell survival by protecting cells from apoptotic signaling mediated by scramblase activity. Together, our data provide substantial evidence showing a novel protective mechanism of TMEM16E protein through the regulation of membrane trafficking system.

Keywords: TMEM16E, Scramblase, Plasma membrane repair (PMR), Acidosis

P-09-016

Neddylation blockade modulates the positive effect of FIH on breast cancer cells.

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FIH (Factor inhibiting HIF-1 α) is a functional protein that inhibits HIF-1 α (Hypoxia-inducible factor-1 α). As a well-known function of FIH, binding to the transcriptional cofactor p300 protein is blocked due to the hydroxylation of the asparagine residue N803 in the C-terminal TAD of HIF-1 α , thereby inhibiting transcriptional activity of HIF-1 α . HIF-1 α is a transcription factor, which mediated for cancer cells to survive low oxygen levels and induces the expression of various genes such as VEGF and CA9.

Interestingly, FIH regulation is very important in preventing cancer metastasis by targeting HIF-1 α . Furthermore, HIF-1 α was found to be affected by neddylation. NEDD8 (Neural precursor cell expressed developmentally downregulated 8) is a process in which the NEDD8 protein binds to the target protein through a series of enzymatic reactions. It is involved in regulating cell growth, viability, and development. Given the previously reported association between FIH and HIF-1 α tumor progression, so we hypothesized that FIH may play an important role in cancer pathogenesis. This study is the first to explain the relationship between neddylation and FIH. We found that inhibition of neddylation in the breast cancer cell enhanced

FIH and revealed that FIH binds to NEDD8. The above results suggest that FIH-mediated NEDD8 and it has a positive effect. Inhibition of neddylation increases the interaction between FIH and HIF-1 α . Owing to this, it will present a new strategy for the development of FIH-targeted cancer and treatment methods.

Keywords: Neddylation, FIH, HIF-1 α

P-09-017

Cancer cells promote lipolysis of adipocyte derived stem cells to obtain free fatty acids for migration by using a cytokine

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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 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 metastasis, Cytokine, Lipolysis, Transcription factor

P-09-018

CRIF1 siRNA-encapsulated PLGA nanoparticles suppress tumor growth in MCF-7 human breast cancer cells

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Mitochondrial oxidative phosphorylation (OXPHOS) system dysfunction in cancer cells has been exploited as a target for anti-cancer therapeutic intervention. The downregulation of CR6-interacting factor 1 (CRIF1), an essential mitoribosomal factor, can impair mitochondrial function in various cell types. In this study, we investigated whether CRIF1 deficiency induced by siRNA and siRNA nanoparticles could suppress MCF-7 breast cancer growth and tumor development, respectively. Our results showed that CRIF1 silencing decreased the assembly of mitochondrial OXPHOS complexes I and II, which induced mitochondrial dysfunction, mitochondrial reactive oxygen species (ROS) production, mitochondrial membrane potential depolarization, and excessive mitochondrial fission. CRIF1 inhibition reduced p53-induced glycolysis and apoptosis regulator (TIGAR) expression and NADPH synthesis, leading to additional increases in ROS production. The downregulation of CRIF1 suppressed cell proliferation and inhibited cell migration through the induction of G0/G1 phase cell cycle arrest in MCF-7 breast cancer cells. Similarly, intratumoral injection of CRIF1 siRNA-encapsulated PLGA

nanoparticles inhibited tumor growth, downregulated the assembly of mitochondrial OXPHOS complexes I and II, and induced the expression of cell cycle protein markers (p53, p21, and p16) in MCF-7 xenograft mice. Thus, the inhibition of mitochondrial OXPHOS protein synthesis through CRIF1 deletion destroyed mitochondrial function, leading to elevated ROS levels and inducing antitumor effects in MCF-7 cells

Keywords: CRIF1, MCF-7 cells, Mitochondrial dysfunction, PLGA, Nanoparticle

P-09-019

Targeted Therapy and anti-PD-1 treatment synergistically promote antitumor immunity in Hepatocellular Carcinoma

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PD-L1 (CD274) is a well-known immunosuppressive molecule expressed by tumor cells to subvert anticancer immunity. By binding to PD1 on T cells, PD-L1 suppresses T cells activation via inhibiting TCR stimulation signaling pathway, leading to T cell dysfunction and tumor immune escape. Although recent studies have published that the expression of PD-L1 has been closely correlated with clinical response to anti-PD1/PD-L1 immunotherapy, the functional role of PD-L1 protein levels in cancers is not well investigated. Posttranslational modifications (PTMs) of PD-L1 have emerged as important regulatory mechanisms that modulate immunosuppression in cancer patients. The role of hematologic and neurological expression 1 (HN1) in cancer has been recently reported and appears to play an important role. However, the relationship between HN1 and immunosuppressive molecules PD1 and PDL1 in HCC is not clearly understood. Here, we suggested that HN1 is a new regulator for PD-L1. This study establishes a key molecular link between targeted therapy and immune surveillance and identifies that combination therapy with HN1 inhibition and an anti-PD-1 antibody has much better antitumor efficacy than either monotherapy in HCC.

Keywords: Hematologic and neurological expression 1 (HN1), Programmed death ligand 1 (PD-L1), Hepatocellular carcinoma (HCC)

P-09-020

Majonoside-R2 active in Vietnamese ginseng has the effect of protecting H9C2 cells against hypoxia/reoxygenation injury via modulating mitochondrial function and biogenesis

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The therapeutic effect on many diseases of Vietnamese ginseng has been known, however, its bioactivity against cardiac hypoxia/reoxygenation (HR) injury remains unclear. In this study, we investigated the protective roles of total saponin extract (TSE) and majonoside-R2 (MR2) targeting mitochondria in HR-induced rat cardiomyocyte H9C2 cells. Interestingly, the results showed that both TSE and MR2 effectively protected the cells from HR damage. Particularly, 9 μ M of MR2 significantly increased the viability of HR-induced cells ($p < 0.05$). We also found that the MR2 treatment markedly prevented the loss of mitochondrial membrane potential and cardiolipin content, and an increase in reactive oxygen species production in HR-treated H9C2 cells. Besides that, MR2 treatment altered the mRNA expression of genes involved in mitochondrial biogenesis under HR conditions. The present study documented for the first time the cardioprotective effects of MR2 against HR injury by mitochondrial function and adjusting mitochondrial

biogenesis.

Keywords: Mitochondria, Majonoside-R2, H9C2 cells, Vietnamese ginseng

P-09-021

Anticancer effect of verteporfin on non-small cell lung cancer via downregulation of ANO1

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Anoctamin 1 (ANO1) is a calcium-activated chloride channel found in various cell types and is overexpressed in non-small cell lung cancer (NSCLC), a major cause of cancer-related mortality. With the rising interest in development of druggable compounds for NSCLC, there has been a corresponding rise in interest in ANO1, a novel drug target for NSCLC. However, as ANO1 inhibitors that have been discovered simultaneously exhibit both the functions of an inhibition of ANO1 channel as well as a reduction of ANO1 protein levels, it is unclear which of the two functions directly causes the anticancer effect. In this study, verteporfin, a chemical compound that reduces ANO1 protein levels was identified through high-throughput screening. Verteporfin did not inhibit ANO1-induced chloride secretion but reduced ANO1 protein levels in a dose-dependent manner with an IC50 value of ~300 nM. Moreover, verteporfin inhibited neither P2Y receptor-induced intracellular Ca²⁺ mobilization nor cystic fibrosis transmembrane conductance regulator (CFTR) channel activity, and molecular docking studies revealed that verteporfin bound to specific sites of ANO1 protein. Confirming that verteporfin reduces ANO1 protein levels, we then investigated the molecular mechanisms involved in its effect on NSCLC cells. Interestingly, verteporfin decreased ANO1 protein levels, the EGFR-STAT3 pathway as well as ANO1 mRNA expression. Verteporfin reduced the viability of ANO1-expressing cells (PC9, and gefitinib-resistant PC9) and induced apoptosis by increasing caspase-3 activity and PARP-1 cleavage. However, it did not affect hERG channel activity. These results show that the anticancer mechanism of verteporfin is caused via the down-regulation of ANO1.

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Keywords: Anoctamin 1, EGFR-STAT3, Verteporfin, Non-small cell lung cancer

P-09-022

Redox function of secreted APE1/Ref-1 downregulates ROS generation and apoptosis in doxorubicin-induced cardiotoxicity

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Background: Doxorubicin is an anthracycline anticancer drug with a potent therapeutic effect against various malignancies. However, its use is limited due to cardiotoxicity, which can lead to heart failure. Apurinic/apyrimidinic endonuclease 1/redox factor-1 (APE1/Ref-1) is a multifunctional protein that regulates oxidative stress and anti-inflammatory function. Secreted APE1/Ref-1 has shown anti-oxidant and anti-inflammatory properties, and its therapeutic potential can be explored.

Purpose: This study aims to test the protective effect of secreted APE1/Ref-1 in the doxorubicin-induced cardiomyopathy model in vivo and in vitro.

Methods: Adenovirus preprotrypsin leading sequence APE1/Ref-1 (AdPPT-LS-APE1/Ref-1), a secretory APE1/Ref-1 adenovirus encoding human APE1/Ref-1 gene, was amplified in HEK293T cells. H9C2 cells were treated with the supernatant of the amplified AdPPT-LS-APE1/Ref-1, and then with 2μM doxorubicin to induce cytotoxicity. In vivo model of doxorubicin-induced cardiotoxicity was established by injecting doxorubicin (15mg/kg) into C57BL/6 mice via the peritoneum. The conditioned media of HEK293T cells with AdPPT-LS-APE1/Ref-1 transfection was injected i.p. 24h before injecting doxorubicin in the experimental group.

Results: Doxorubicin caused apoptosis and oxidative stress in H9C2 cells and cardiomyopathy in mice. NT-proBNP level increased both in vitro (p<0.001) and in vivo (p<0.05) upon administering doxorubicin. Interestingly, doxorubicin increased p53 level in a dose-dependent manner in 48 hr, but the cellular APE1/Ref-1 level did not change. Secreted APE1/Ref-1 in the conditional media showed redox activity and decreased NT-proBNP in doxorubicin-treated H9C2 cells and mice (p<0.001 and p<0.05, respectively). Doxorubicin aggravated cell death (p<0.001) and oxidative tissue damage (p<0.001) in H9C2 cells, while pre-treatment of secreted APE1/Ref-1 inhibited cell death in H9C2 cells (p<0.05). Secreted APE1/Ref-1 significantly reduced the expression of p53 (p<0.001) and its effectors, Bax/Bcl-2 (p<0.05) and Caspase-3 (p<0.05) in doxorubicin-treated H9C2 cells. It also reduced p53 (p<0.05) along with Bax/Bcl-2 (p<0.05) and Caspase-3 (p<0.05) in doxorubicin-treated mice (n=4 in each group). Finally, secreted APE1/Ref-1 significantly decreased intracellular ROS production (p<0.001).

Conclusion: Redox function of secreted APE1/Ref-1 is protective against doxorubicin-induced cardiotoxicity via suppressing ROS generation and apoptosis.

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Keywords: APE1/Ref-1, Doxorubicin, Cardiotoxicity, Heart failure, Redox

P-09-023

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) can transform into myofibroblast by noxious stresses, which play a pathogenic role in liver fibrosis. Transforming growth factor-β (TGF-β) and integrin signaling are known to involve in HSC activation; however, its molecular mechanism remains unclear. We used isolated primary HSCs from balb/C mice and thioacetamide (TAA)-injected fibrosis mouse model. Culturing on a polystyrene surface itself activated quiescent HSCs within 7 days and increased TGF-β expression and secretion, which are abrogated by blocking integrin receptor activation. Autocrine action of TGF-β triggered Smad-2/3-ERK1/2-mTOR-NOX4 activation, ROS generation, and fibrogenesis, all of which are prevented by TGF-β blocking antibody or receptor II blockers. Extra- or intracellular Ca²⁺ chelation, as well as Ca²⁺ channel blockers, suppressed TGF-β/integrin signaling and fibrotic changes, suggesting the exclusive Ca²⁺-dependence on HSC activation. Notably, α-Klotho interfered TGF-β receptor activation and prevented TGF-β/integrin signaling, oxidative stress, cytosolic Ca²⁺ elevation, and transdifferentiation of HSCs in vitro. Furthermore, in vivo application of α-Klotho significantly alleviated TAA-induced liver fibrosis and damage. These results suggest that targeting TGF-β/integrin signaling via α-Klotho could be novel therapeutic strategy against chronic fibrotic diseases.

Keywords: Hepatic stellate cells, Fibrosis, TGF-β, Integrin receptor, Klotho

P-09-024

Down-regulation of TASK-3 induces senescence of granulosa cells in the bovine follicular cystic ovaryChang-Woon Kim¹, Eun-Jin Kim², Min Seok Woo², Dang Long Cao^{2,3}, Ji Hyeon Ryu⁴, IL-Keun Kong⁵, Dong Kun Lee^{2,3}, Seong-Geun Hong², Jaehee Han², **Dawon Kang**^{2,3}¹Department of Obstetrics and Gynecology, Samsung Changwon Hospital, Sungkyunkwan University School of Medicine, ²Department of Physiology and Institute of Health Sciences, College of Medicine Gyeongsang National University, ³Department of Convergence Medical Science Gyeongsang National University, ⁴Research Institute for Convergence of Biomedical Science and Technology Pusan National University Yangsan Hospital, ⁵Division of Applied Life Science (BK21 Plus) Gyeongsang National University

Ovarian cysts, which can be associated with infertility in females, result from various factors, including imbalanced hormone levels and gene expression profiles. TWIK-related acid-sensitive K⁺ (TASK) channels, members of the two-pore domain K⁺ (K2P) channel family, are expressed in mammalian reproductive cells and underlie K⁺ efflux related to osmotic water movement. This study was conducted to identify whether TASK expression is changed in cystic ovaries and, if so, what its role is. Ion concentration and TASK expression level were analyzed in follicular fluid (FF) and granulosa cells (GCs) obtained from small-sized (5 to 10 mm in diameter) and large-sized (> 25 mm) follicles (SF and LF) in Korean cattle. The concentrations of K⁺, Na⁺ and Cl⁻ in FF obtained from SF (SFFF) were 10.4 ± 3.5 mEq/L, 138 ± 11.9 mEq/L, and 104.9 ± 7.0 mEq/L, respectively. However, the K⁺ concentration was significantly decreased in LFFF (6.2 ± 0.8 mEq/L; p < 0.05). Semi-quantitative PCR, western blot, and immunocytochemistry data showed that the TASK-3 expression level significantly decreased by approximately 50% in both LF and LFGCs compared to the corresponding controls. The TASK-3 protein was localized at the plasma membrane of GCs. The size of LFGC in diameter was larger than SFGC (16.6 ± 0.7 μm in SFGC versus 22.7 ± 1.2 μm in LFGC). The cell swelling in response to exposure to the hypotonic solution (200 mOsm/L) was highly decreased in TASK-3-overexpressed cells compared to vector-transfected cells. TASK-3-knockdown cells showed arrested growth without cell death. Senescence markers were highly detected in LFGC and TASK-3-knockdown cells. These results showed that low TASK-3 expression in LF is associated with the arrest of GC growth and oocyte maturation, leading to senescence of GCs and cyst formation.

Keywords: Cyst, Granulosa cell, Senescence, TASK

P-09-025

Isolation and analysis of circulating exosomes in a mouse model of metastatic lung cancer**Kang Minji**¹, Lim Jiwoo¹, Ahn Young-Ho², Cho Min-Sun³, Lee Kang Jihee¹, Choi Youn-Hee¹¹Physiology Inflammation-Cancer Microenvironment Research Center, College of Medicine, Ewha Womans University, Seoul, South Korea, ²Molecular Medicine Inflammation-Cancer Microenvironment Research Center, College of Medicine, Ewha Womans University, Seoul, South Korea, ³Pathology College of Medicine, Ewha Womans University, Seoul, South Korea

Lung cancer is the leading cause of cancer-related death worldwide. Many patients with advanced lung cancer develop brain metastasis (BM); however, the mechanism by which cancer cells break away from the original tumor and spread to brain is not clearly known. Recent studies have shown that tumor-derived exosomes and exosomal proteins contribute to establish a metastatic niche within distant sites, away from the primary tumor, by intercellular communication between tumor cells and stromal cells in distant microenvironment. The purpose of this study was to identify the biomarkers and potential therapeutic targets for cancer progression and metastasis by analyzing the circulating exosomes in a syngeneic lung cancer mouse model. We performed exosome protein analysis to compare the protein expression levels that change during cancer progression and metastasis

in lung cancer. We found that the expression level of vinculin protein, a direct target of miR-34 family members, was significantly increased in serum exosome samples from a mouse model of advanced lung cancer with BM compared to samples from early cancer without BM. In addition, we found that the levels of exosomal vinculin and number of tumor metastases were significantly reduced in syngenic mice injected with miR-34-transduced lung cancer cells. These results demonstrate that circulating exosomes can serve as diagnostic and prognostic markers as well as therapeutic targets for predicting and preventing tumor metastasis.

Keywords: Lung cancer, Brain metastasis, Exosome, MiR-34 family

P-09-026

TREK-1 upregulation promotes the growth of colorectal cancer cells along with PDGFRα activation**Min Seok Woo**¹, Young-Tae Ju², Eun-Jin Kim¹, **Dawon Kang**¹¹Department of Physiology and Institute of Health Sciences College of Medicine, Gyeongsang National University, ²Department of Surgery College of Medicine, Gyeongsang National University

Colorectal cancer (CRC) is a leading cause of morbidity and mortality worldwide due to the lack of reliable biomarkers for early detection. Platelet-derived growth factor (PDGF) and PDGFR receptor alpha (PDGFRα) have a role in oncogenesis, and PDGF regulates gene transcription by binding to its specific receptors. Cells expressing PDGFRα (PDGFRα+) highly express TWIK-related K⁺ (TREK-1), a member of the two-pore domain K⁺ channel. This study was performed to determine the role of TREK-1 and its association with PDGFRα in the oncogenesis of CRC. Expression Atlas and The Human Protein Atlas showed that the survival rate of CRC patients with a high TREK-1 expression level was lower than those with a common TREK-1 expression. PDGFRα and TREK-1 expression levels were higher in human CRC than in normal tissues. In colon cancer cell lines (HCT116 and SW620) overexpressed with TREK-1, cell proliferation, migration, and spheroid growth were significantly increased (p < 0.05). The expression level of PDGFRα, VEGF, and cyclin D1 and the activation of AKT, ERK, and STAT3 were markedly increased in TREK-1-overexpressed cells compared with vector-transfected cells. These results showed that TREK-1 is associated with PDGFRα in the tumorigenesis of CRC and suggest that TREK-1 and PDGFRα may be helpful biomarkers for CRC.

Keywords: Colorectal cancer, PDGFRα, TREK-1

P-09-027

Drug discovery for overcoming acquired resistance to ALK inhibitors in lung cancer based on a systems approach**Sang-Min Park**¹, Haejeong Heo^{2,3}, Hyun Jung Lim^{2,3}, Seongwon Cha⁴, Mirang Kim^{2,3}, **Haeseung Lee**⁵¹College of Pharmacy Chungnam National University, ²Personalized Genomic Medicine Research Center Korea Research Institute of Bioscience and Biotechnology (KRIBB), ³Department of Functional Genomics University of Science and Technology (UST), ⁴Korean Medicine (KM) Data Division Korea Institute of Oriental Medicine (KIOM), ⁵College of Pharmacy Pusan National University

Anaplastic lymphoma kinase (ALK) inhibitors (ALKi) are effective in treating lung cancer patients with chromosomal rearrangement of ALK. However, after continuous treatment with ALKi, cancer cells invariably acquire resistance to the drugs. Here, we propose an efficient strategy to suppress ALKi resistance based on a meta-analysis of transcriptome data derived from cell models of acquired resistance to ALKi. We systematically identified gene signatures with expression patterns that were consistently altered during the development of resistance to ALKi. Using the resistance signatures, we conducted in silico drug screening to identify novel compounds that are likely to reverse the expression levels of the resistance signatures.

Three compounds, brefeldin A, emetine, and SB-743921, were predicted as promising candidate drugs to inhibit the growth of cells resistant to ALKi. These candidate drugs were demonstrated to be effective in inhibiting the growth of ALKi-resistant cells, and the clinical relevance of the resistance signatures was interpreted. Our transcriptome-guided systems approach paves the way for efficient drug discovery for overcoming acquired resistance to cancer therapy.

Acknowledgement: A portion of the data used for this study was obtained from the Genome-InfraNet (IDs: 1711048605, 1711048587, 1711041874, 1711041928, and 1711072542) of the Korea Bioinformatics Center.

Keywords: ALK inhibitor, Acquired resistance, Transcriptome, Connectivity map, Drug repositioning

P-10-001

A comparison of metabolic profile and cardiorespiratory fitness of breast cancer survivors and matched healthy controls.

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Purpose: Nonalcoholic fatty liver disease (NAFLD) is linked to breast cancer risk and recurrence. However, it is unclear whether the increased NAFLD risk parameters in breast cancer survivors (BCS) are attributable to breast cancer or simply to a general trend among middle-aged women. In addition, there was a lack of evidence of the association between fitness level and NAFLD parameters in BCS.

Thus, this study aimed to compare the NAFLD risk parameters between women without breast cancer (non-BCS) and BCS as well as the association between NAFLD risk parameters and fitness level in BCS.

Methods: Using a case-control design, women with treated stage <4 breast cancer (n=40) and non-BCS women (n=40) were matched 1:1 by age and weight. We measured body composition, NAFLD-related biomarkers, and fitness parameters. We also calculated Fatty Liver Index (FLI) which is a non-invasive method for predicting NAFLD. Data analysis was performed using SPSS 26 software, and statistical significance was set at P<0.05.

Results: The mean age was 54.2 years in BCS and 54.3 in non-BCS. 57.5% of BCS were diagnosed with cancer stage <2, and 45% were diagnosed within 3 years of their breast cancer diagnosis. There was no significant difference in characteristics between groups. Compared to non-BCS, BCS had significantly higher body fat percentages (p<0.001), fasting glucose (p<0.05), insulin (p<0.05), HOMA-IR (p<0.05), AST (p<0.01), and WISP-1 (p<0.05). WISP-3 (p<0.05) and cardiorespiratory fitness (p<0.05) in BCS were significantly lower than in non-BCS. In particular, BCS with high FLI (≥30) showed significantly worse body composition, NAFLD-related biomarker parameters, and fitness than BCS with low FLI (<30). However, even with high FLI levels, BCS with high cardiorespiratory fitness had significantly lower BMI (p<0.01), insulin (p<0.05), and FLI (p<0.05) than BCS with low cardiorespiratory fitness.

Conclusion: BCS have a higher risk than non-BCS of developing NAFLD risk factors. BCS should take care to manage their fat and fitness levels to lower their risk of metabolic disturbances.

Keywords: Breast cancer, Nonalcoholic fatty liver disease (NAFLD), Cardiorespiratory fitness

P-10-002

Chronic food restriction produces locomotor sensitization to amphetamine

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Ghrelin, a hormone derived from the stomach, mainly functions to increase appetite. Interestingly, it also affects reward-seeking and addictive behaviors. We have previously demonstrated that microinjection of ghrelin into the nucleus accumbens (NAcc) core produces locomotor sensitization in amphetamine (AMPH) pre-exposed rat, when dopamine D1 receptor is co-activated. Here, we further investigated whether actual food restriction by itself may produce similar effects. Rats were housed with food pellets, either normal or restricted (acute or chronic). With this procedure, plasma ghrelin concentration was found to be significantly increased only in chronic food-restricted rats. After 2 weeks, when their locomotor activities were measured following challenge with AMPH (1 mg/kg, IP), only chronic food-restricted rats produced sensitized-locomotor activity. These results indicate that food restriction by itself is sufficient to produce locomotor sensitization to AMPH. We further examined whether there are any differential molecular changes in the NAcc under the chronic food-restriction conditions following an AMPH challenge, which will be discussed in the present presentation.

Keywords: Food restriction, Ghrelin, Nucleus accumbens, Amphetamine, Locomotor sensitization

P-10-003

Effects of exercise training on mitochondrial dysfunction associated with aging

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Skeletal muscle dysfunction associated with aging can contribute to increased mortality and decreased quality of life. Exercise training can be an efficient, non-pharmacological way to maintain skeletal muscle function in the setting of aging. Mitochondrial function is one of the most important indices to influence skeletal muscle function, and previous studies have shown that mitochondrial function decreases with aging. Nevertheless, the effects of exercise training on mitochondrial dysfunction associated with aging have not been fully understood. This study aims: first, to investigate the changes in mitochondrial function with aging and second, to evaluate the effect of endurance exercise training on two different skeletal muscles (extensor digitorum longus and soleus) from aged male C57BL/6 mice. As a result, enzyme activities involved in the TCA cycle and electron transport chain reaction declined in skeletal muscles of aged mice compared to young mice, while these age-associated decreases in enzyme activities were ameliorated/prevented by exercise training. The aging and exercise training effects are not fiber-type specific in most cases. Additionally, the transcriptional activation of genes known to be associated with the mitochondrial function was altered with aging, while exercise training mitigated/reversed these aging-associated changes. Conclusively, these findings suggest that exercise training can mitigate the age-associated decline in mitochondrial function, and these effects might not be fiber-type specific in mouse skeletal muscles.

Keywords: Aging, Exercise training, Mitochondrial dysfunction, Muscle fiber types

P-10-004

Neddylolation attains bone homeostasis by regulating osteoclastogenesis and osteoblastogenesisJoseung Lee¹, [Min Young Lee](#)¹, Yang-Sook Chun^{1,2,3}¹Department of Biomedical Sciences Seoul National University College of Medicine, Seoul, Korea, ²Ischemic/Hypoxic Disease Institute Seoul National University College of Medicine, Seoul, Korea, ³Department of Physiology Seoul National University College of Medicine, Seoul, Korea

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Recently, neddylation, a post-translational modification involved in regulating various activities of target proteins, 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. Here, we demonstrate that neddylation inhibition bidirectionally regulates bone formation controlling both osteoclastogenesis and osteogenesis. We show the molecular mechanism regarding the process of neddylation for both Nuclear Factor Of Activated T Cells 1 (Nfatc1) and Runt-related transcription factor 2 (RUNX2), which are key regulators for osteoclast and osteoblast differentiation, respectively. Neddylation increased transcriptional activity of Nfatc1 while promoting ubiquitin-mediated proteasomal degradation in Runx2, showing a reciprocal role in bone homeostasis. Taken together, this study indicates that neddylation may contribute in accomplishing bone homeostasis and providing a novel therapeutic strategy for osteoporosis treatment.

Keywords: Neddylation, Nfatc1, RUNX2, Osteoclastogenesis, Osteogenesis

P-10-005

Association between body mass index, domain-specific sedentary behavior, and asthma risk by using Korean Youth Health Risk Behavior Online Survey[Ki-Taek Oh](#), Jihee Min, In Deok Kong

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Background: Proper physical activity may affect positive results on asthma. On the other hand, the association between sedentary behavior and asthma risk is not fully understood. Furthermore, obesity is one of the majority risk factors in asthma incidence, but subjective body image and asthma risk have not been elucidated. Thus, this study investigates the relationship between domain-specific sedentary behavior, body mass index (BMI), body image, and the prevalence of asthma in Korean adolescents.

Methods: The 16th and 17th Korean Youth Health Risk Behavior Online Surveys (2020-2021) were used, and 6,452 respondents aged between 14-19 years were analyzed. The sedentary behavior consists of study-time and leisure-time sedentary behavior, and we divided each domain as high/low based on the 50 percentiles. Body image consists of 5 categories (i.e., very obese, obese, normal, thin, and very thin). The characteristics analysis between asthma and non-asthma group was conducted using Chi-square analysis and ANCOVA analysis. In comparing the relationship between Asthma and sedentary behavior, BMI, and body image, it was expressed as odds ratio and 95% confidence interval using logistic regression analysis

Results: Adolescents who were in high leisure-time sedentary behavior groups showed a significant increase in asthma risk up to 1.224 (95%CI: 1.073-1.396) times that of adolescents with low sedentary behavior. In addition, adolescents who were overweight/obese or perceived their body image as obese showed 1.268 times (95%CI: 1.092-1.472), 1.295 times (95%CI: 1.139-1.473) higher asthma risk, respectively, than those who did not. Considering both leisure-time sedentary behavior and obesity, adolescents who were overweight/obese and had high leisure-time sedentary behavior groups showed a significant increase in asthma risk up to 1.446 (95%CI: 1.170-1.787). adolescents who had fat body image and had high leisure-time sedentary behavior groups showed a significant increase in asthma risk up to 1.559 (95%CI: 1.295-1.878).

Conclusion: These findings suggest that adolescent students with more leisure-time sedentary behavior have more asthma risk than those with less leisure time sedentary behavior. Furthermore, odds ratio got higher combining obesity with leisure-time sedentary behavior.

Keywords: Adolescence, Asthma, Sedentary behavior, Obesity, Body image

P-10-006

Novel Function of Jumonji C (JmjC) domain – containing protein in osteoclastogenesis[Joo-Seung Lee](#)¹, Hye-Jin Kim¹, Min Young Lee¹, Seon-Young Kim¹, Do-Won Jeong¹, Jong-Wan Park^{1,2,3}, Yang-Sook Chun^{1,2,3}¹Department of Biomedical Sciences Seoul National University, ²Ischemic/Hypoxic Disease Institute Seoul National University, ³Department of Physiology Seoul National University College of Medicine, Seoul, Korea

The regulation of osteoclastogenesis is critical to maintain physiological bone homeostasis and prevent bone-destructive diseases. The nuclear factor of activated T-cells calcineurin-dependent 1 (NFATc1) plays an essential role in osteoclastogenesis, and its expression is induced during early osteoclastogenesis.

On the other hand, the Jumonji C (JmjC) domain-containing protein (JHDM), a histone demethylase, catalyzes histone 3 lysine 9 and is involved in osteoblastic bone formation. However, the mechanism for regulation of the enzymatic activity of JHDM in osteoclastogenesis is not yet well known. Here, we show that JHDM is a key negative regulator during receptor activator of nuclear factor- κ B ligand (RANKL)-induced osteoclastogenesis. The expression level of JHDM gradually decreased during osteoclastogenesis in bone marrow macrophages (BMMs) treated with RANKL. Down-regulated expression of JHDM strongly facilitated osteoclast formation together with induction of several osteoclast-specific genes such as TRAP, Oscar and CathepsinK. NFATc1 proteins are ubiquitinated and rapidly degraded during late stage osteoclastogenesis. Interestingly, overexpression of JHDM induces NFATc1 degradation during late stage osteoclastogenesis. Taken together, the present study demonstrated that JHDM is a post-translational co-repressor for NFATc1 that attenuates osteoclastogenesis.

Keywords: Osteoclastogenesis, JHDM, NFATc1, Post-translational modification

P-10-007

Exercise training reduces a high-fat diet-induced CXCL12 expression in mouse[Elsayed Mohamed](#)^{1,2}, Dong-Hwan Kim³, Bong-Jo Kim¹, Hae-Rahn Bae¹¹Department of Physiology College of Medicine, Dong-A University, Busan, Korea,²Department of Genetics Assiut University, Assiut, Egypt, ³Human Life Research Center Dong-A University, Busan, Korea

Obesity is a low level of chronic inflammation that induces the release of lipids, aberrant adipokines, pro-inflammatory cytokines, and chemokines from adipose tissue. CXCL12, an adipocyte-derived chemotactic factor, plays an important role in inducing insulin tolerance by recruiting macrophages, lymphocytes, and hematopoietic cells into adipose tissues. Exercise training is well known to improve adipose tissue metabolism and insulin sensitivity. In the present study, we investigated the effects of high-fat diet (HFD) and exercise training on the expression of CXCL12 in different types of adipose tissues, and its plasma concentration. Adult male CD1 mice were fed with HFD for four and eight weeks (HFD4 and HFD8 groups, respectively), followed by treadmill exercise training for another four and eight weeks (HFD4T and HFD8T groups, respectively). The mice fed with HFD exhibited 20.9% and 28.9% body weight gain after four and eight weeks, respectively, compared to the control value of 10.4%. Treadmill exercise reduced body weight gain to 18.2% and 19.4% for four and eight weeks, respectively. The genital, inguinal and interscapular fat depots were enlarged along with an

increase in the number and the mean size of adipocytes after a high-fat diet, which was remarkably reduced by exercise training. Relative weights of total fat tissues increased by 1.3 and 1.7 folds in HFD4 and HFD8 groups, respectively, compared to the control, and reduced by 1.5 and 2.0 folds in HFDT4 and HFDT8 groups, respectively, compared to corresponding each HFD group. Cytokine array and ELISA analysis revealed a significant increase of plasma CXCL12 after HFD as well as a return to the control value by exercise training. Immunofluorescence analysis confirmed that the HFD-induced expression of CXCL12 in adipose tissues was alleviated by exercise training. Taken together, exercise training is efficient to reduce HFD-induced CXCL12 secretion from adipose tissues, and helps to overcome obesity complications involved with CXCL12-mediated adipose tissue inflammation and insulin resistance.

Keywords: Exercise, High-fat diet, Obesity, CXCL12, Mouse

P-11-001

Simulation of substrate-dependent changes of mitochondrial function using a computational mitochondria model

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Mitochondrial substrates are essential for generation of ATP in the process of oxidative phosphorylation. They enter the citric acid cycle to generate NADH and FADH₂ which are the source of electrons in the oxidative phosphorylation. A set of substrates which can maximize the mitochondrial respiration, however, is still in pursuit.

We constructed a computational interface to simulate the effects of substrate composition. The model of mitochondria includes the citric acid cycle, electron transport system, substrate transporters, and malate-aspartate shuttle. We tested the effects of substrate combination in the cytosolic space on the mitochondrial generation of NADH. We compared those effects between permeabilized ventricular myocytes and computation model. In case of single substrate, only pyruvate slightly increased the NADH in both permeabilized ventricular myocytes and computational model. In case of double substrate, either combination of malate/pyruvate or pyruvate/succinate increased the NADH in permeabilized ventricular myocytes whereas combination of malate/pyruvate or malate/glutamate increased the NADH in the computational model. In case of triple substrate, all the combinations except glutamate/succinate/malate increased the NADH in permeabilized ventricular myocytes whereas all the combinations except glutamate/succinate/pyruvate increased the NADH in the computational model. In case of using all substrates, it increased the NADH in both the permeabilized ventricular myocytes and computational model.

In conclusion, our computational model would be very useful for both the educational tool and research aid though it still needs to be remedied to better simulate the experimental results.

Keywords: Mitochondria, Simulation, Substrate, Computational model

P-12-001

Prediction of the Medicinal Mechanisms of *Pinellia ternata* Breitenbach, a Traditional Medicine for Gastrointestinal Motility Disorders, through Network Pharmacology

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Pinellia ternata Breitenbach (PTB) is a widely used herbal medicine in China, Japan, and South Korea. It has antiemetic, anti-inflammatory, antitussive, and sedative properties. The raw material is toxic, but can be made safer using alum solution or by boiling it for a long time. In addition, PTB seems to be effective for gastrointestinal motility disorders (GMDs), but this is yet to be conclusively proven. Herein, PTB compounds, targets, and related diseases were investigated using the traditional Chinese medical systems pharmacology database and an analysis platform. Information on target genes was confirmed using the UniProt database. Using Cytoscape 3.8.2, a network was established and GMD-related genes were searched using the Cytoscape stringApp. The effects of the PTB extract on the pacemaker potential of interstitial cells of Cajal and GMD mouse models were investigated. In total, 12 compounds were found to target 13 GMD-related genes. In animal experiments, PTB was found to better regulate pacemaker potential in vitro and inhibit GMD signs compared to control groups in vivo. Animal studies showed that the mechanism underlying the effects of PTB is closely related to gastrointestinal motility. The results obtained demonstrated that PTB offers a potential means to treat GMDs, and we suggested that the medicinal mechanism of GMDs can be explained by the relationship between 12 major components of PTB, including oleic acid, and 13 GMD-related genes.

Keywords: *Pinellia ternata* Breitenbach, Gastrointestinal motility disorders, Network-based systems pharmacological, Traditional medicine

P-12-002

Measuring Pattern Separation in Hippocampus by in Situ Hybridization

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Distinguishing different contexts is thought to involve a form of pattern separation that minimizes overlap between neural ensembles representing similar experiences. Theoretical models suggest that the dentate gyrus (DG) segregates cortical input patterns before relaying its discriminated output patterns to the CA3 hippocampal field. This suggests that the evaluation of neural ensembles in DG and CA3 could be an important means to investigate the process of pattern separation. In the past, measurement of entorhinal cortex (EC), DG, and CA3 ensembles was largely dependent upon in vivo electrophysiological recording, which is technically difficult. This protocol provides a method to instead measure pattern separation by a molecular method that provides direct spatial resolution at the cellular level.

Keywords: Ensemble, Hippocampus, Homer1a, Pattern separation

P-12-003

Targeted downregulation of Hipp1 ameliorates tau-induced deficits in *Drosophila melanogaster*Sung Yeon Park^{1,3}, Jieun Seo², Seulbee Lee², Joohyung Kim⁴, Sang Jeong Kim^{1,2,3}, Seungbok Lee⁴, Yang-Sook Chun^{1,2,3}¹Ischemic/Hypoxic Disease Institute Seoul National University College of Medicine,²Department of Biomedical Sciences Seoul National University College of Medicine,³Department of Physiology Seoul National University College of Medicine,⁴Department of Brain and Cognitive Sciences Seoul National University

Background: Tauopathies, such as Alzheimer's disease (AD), are neurodegenerative diseases characterized by the deposition of neurofibrillary tangles comprising hyperphosphorylated tau protein in the human brain. Given that abnormal epigenetic alterations in heterochromatin configuration have been documented in AD patients and transgenic animal models of AD, we investigated the roles of novel heterochromatin-associated interactors in tauopathies.

Methods: We examined whether tissue-specific downregulation or loss-of-function alleles of heterochromatin-associated interactors can affect tau-induced neurotoxicity using transgenic flies via UAS-Gal4 binary system.

Results: Here, we found that knockdown of HP1 and insulator partner protein (Hipp1) ameliorates tau-induced eye defects, locomotion defects, reduced lifespan, and neurodegeneration. Nonetheless, RNAi-mediated reduction of Hipp1 failed to restore tau-induced heterochromatin loosening; it accelerated abnormal overexpression of heterochromatic genes. Instead, knockdown of Hipp1 restored tau-driven aberrant expression of putative insulator targets and aberrant insulator-mediated epigenetic alterations. Hipp1 may have a role as an insulator binding partner regarding to be implicated in tau-induced neurodegeneration. Moreover, knockdown of Hipp1 in flies overexpressing tau restored the aberrant expression of AD susceptibility genes, *Amph* and *Sox102F*.

Conclusion: These results suggest that downregulation of Hipp1 expression may be a potential therapeutic target in neurodegenerative diseases; they also provide new insights regarding the roles of insulator proteins in tauopathies.

Keywords: *Drosophila melanogaster*, Tauopathy, Hipp1, Heterochromatin, Insulator

P-12-004

A new approach of electrophysiologic efficacy evaluation method for APP/PS1 transgenic miceYoungHwan Kim^{1,2}, Ji-Hyun Jeong¹, Ji Woong Ahn¹, Seungsoo Chung^{1,2}¹BnH Research Co., Ltd. Research Institute, ²Department of physiology Graduate School of Medical Science, Brain Korea 21 Project, Yonsei University College of Medicine

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).

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.

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: Alzheimer's disease, Thalamocortical, Long-term potentiation, E/I balance, Potency

P-12-005

Novel marine compound Neopetroside A confers cardioprotection against ischemia/reperfusion injury by inhibiting GSK-3 β Jubert Marquez^{1,2}, Hyoung Kyu Kim^{1,2}, Min Kim^{1,2}, Nikolay Nifantiev³, Jin Han^{1,2}¹Cardiovascular and Metabolic Disease Center Inje University, Busan, Korea, Republic of Korea, ²Department of Health Sciences and Technology Graduate School, Inje University, Busan, Korea, Republic of Korea, ³GB Elyakov Pacific Institute of Bioorganic Chemistry Far Eastern Branch of the Russian Academy of Science, Vladivostok, Russia

Background and Objective: Recent trends suggest novel natural compounds as promising treatments for cardiovascular disease. The authors examined how neopetroside A (NPS A), a natural pyridine nucleoside containing an α -glycoside bond, regulates mitochondrial metabolism and heart function, and investigated its cardioprotective role against ischemia/reperfusion (I/R) injury.

Methods: 8 week-old male Sprague Dawley rats were anesthetized and hearts were excised. Each rat heart was used to measure ex vivo cardiac hemodynamics, infarct size, and for isolating cardiomyocytes and mitochondria. For the myocardial infarction (MI) model, 8-week-old C57BL/6 mice were anesthetized, and sham and MI surgeries were performed. After surgery, mice were injected with 3 mg/kg of NPS A or PBS every two days for four weeks. For in vivo treatment of compounds, 3 mg/kg of NPS A, SB216763, or a combination of both SB216763 and NPS A was administered to 8-week-old C57BL/6 mice every two days for a total of four weeks. Rat cardiomyoblast H9c2 cells were cultured in DMEM with 10% FBS, and penicillin/streptomycin before being treated with compounds. Cell viability, NAD⁺/NADH ratio, mitochondrial function and parameters, and western blot analysis were performed afterward. Autodock 4.01 was used for docking simulations. Direct target binding of NPS A was assessed using surface plasmon resonance.

Results: NPS A reduced ex vivo I/R-induced damage in rat hearts by preserving hemodynamics and mitochondrial respiration capacity. Furthermore, NPS A significantly suppressed cardiac fibrosis in vivo after MI. Further in vitro investigation revealed 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-3 β . Docking simulation studies and surface plasmon resonance binding assays revealed interactions of NPS A with GSK-3 β . Furthermore, NPS A increased the NAD⁺/NADH ratio via the Nrf2/Nqo1 axis, revealing a mechanism underlying the effects of NPS A on metabolic and cellular processes.

Conclusions: NPS A enhances mitochondrial metabolism and protects the heart against I/R damage by inhibiting GSK-3 β . The combined effects of NPS A may serve as a potential treatment against acute I/R damage and myocardial fibrosis

Keywords: NPS A, Marine pyridine α -nucleoside, Mitochondria, Ischemia/reperfusion injury, GSK-3 β inhibition

P-12-006

Mitochondrial creatine kinase tyrosine residue phosphorylation attenuate cardiac hypoxia/reoxygenation injury

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Rationale & Objective: Ischemic cardiomyopathy (ICM) remains a major cause of heart failure-related mortality worldwide. Ischemic preconditioning (IPC), short periods of ischemia followed by a prolonged ischemia/reperfusion (I/R) injury, is one modality that is effective in alleviating ICM. Mitochondria play a significant role in heart disease progression, thus a good target for ICM treatment. In this study, we focused on mitochondrial creatine kinase (CKMT2) under I/R injury.

Methods: Ex vivo Langendorff system of Sprague-Dawley rat hearts simulated normal perfusion, I/R and IPC conditions followed by phosphoproteomic analyses for mitochondrial protein targets. Human cardiomyocyte cell line AC16 was used for in vitro study to define the cardioprotective role of CKMT2.

Results: CKMT2 was dephosphorylated during ischemia and I/R but remained phosphorylated upon IPC conditions. CKMT2 overexpression increased cell viability and mitochondrial ATP level against hypoxia/reoxygenation. Conversely, CKMT2 phosphomutation decreased cell viability and increased ROS during H/R. Increased mitochondrial function via the PGC-1 α /ERR α pathway was observed upon CKMT2 overexpression.

Conclusion: These results suggest that regulation of quantitative expression and phosphorylation site Y368 of CKMT2 offers a unique mechanism in future ICM therapeutics.

Acknowledgement: This research was supported by the Basic Research Lab Program and the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Korean government Ministry of Science and ICT (NRF2020R1A4A1018943, 2018R1A2A3074998).

Keywords: Creatine kinase, Hypoxia, Reoxygenation, Mitochondria, Phosphorylation

P-12-007

Chrysosplenol C potently decreases mitochondrial reactive oxygen species independently of protein kinase C

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Chrysosplenol C (4',5,6-trihydroxy-3,3',7-trimethoxyflavone) is a flavone contained in several medicinal plants including *Milium balansae*. We previously reported that this chemical increases cardiac myocyte contractility via enhancing Ca-induced Ca release and Ca sensitivity of Ca release sites (ryanodine receptor type 2 clusters). One of mechanisms for Ca release sites sensitization is oxidation of the ryanodine receptors in cardiac cells. Therefore we examined if chrysosplenol C modulates the level of reactive oxygen species (ROS) in cardiac myocytes. We monitored mitochondrial ROS (miROS) with the fluorescence dye Mito-SOX using real time confocal imaging in isolated rat ventricular myocytes. We found that chrysosplenol C significantly reduces the level of miROS in a concentration-dependent manner with an EC₅₀ of ~35 μ M. Compared with well-known miROS scavenger mito-TEMPO, the maximal ROS reducing effect by chrysosplenol C was somewhat stronger at its maximally effective concentrations (~150 μ M). Such miROS lowering effect was maintained and somewhat enhanced when cellular superoxide dismutase was inhibited. Because chrysosplenol C-mediated enhancement

of Ca releases are inhibited by protein kinase C (PKC) inhibition we tested if miROS lowering effect by chrysosplenol C is linked to PKC modulation. In the cells preincubated with PKC inhibitors, GF109203X or chelerythrine, chrysosplenol C still decreased miROS to the similar extents. It was noted that GF109203X, but not chelerythrine, significantly increased miROS by itself. Our data suggest that chrysosplenol C is a strong miROS lowering agent in cardiac myocytes, and that its positive inotropic effect may not be linked to its ROS lowering efficacy.

Keywords: Mitochondrial ROS, Chrysosplenol C, Cardiac myocytes, PKC

P-12-008

Multi-modal effects of Echinochrome A on the activities of ion channels in skin tissue

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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 Ca²⁺ 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 IC₅₀ of 2.1 and 2.4 μ M 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 partly inhibited 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 Ca²⁺ 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

P-12-009

Binding mechanisms of Shikonin derivatives targeting SARS-CoV-2 main proteaseRaju Das¹, Yohan Seo², [JooHan Woo](#)^{1,3}¹Department of Physiology Dongguk University College of Medicine, Gyeongju, the Republic of Korea, ²New Drug Development Center Daegu Gyeongbuk Medical Innovation Foundation, Daegu, the Republic of Korea, ³Channelopathy Research Center (CRC) Dongguk University College of Medicine, Goyang, Gyeonggi-do, the Republic of Korea

Shikonin, a well-known bioactive chemical present in the dried roots of *Lithospermum erythrorhizon*, is recognized for its broad-spectrum activities against cancer, oxidative stress, inflammation, virus, and anti-COVID-19 agent. In recent discovery, the crystallographic study of Shikonin bound Main protease (Mpro) of SARS CoV-2 revealed different conformation, suggesting the possibility of designing Shikonin derivatives as potential inhibitors of Covid. Towards the goal, the present study is carried out to explore insights of Shikonin derivatives and find out the possibility of Shikonin derivatives targeting Mpro of Covid by using molecular docking and molecular dynamics simulation. A total of 25 Shikonin derivatives are introduced in this study, from where seven derivatives showed higher binding affinity than Shikonin, while four derivatives obtained the highest binding energy in MM-GBSA binding energy calculation. According to Molecular dynamics simulation results, selected Shikonin derivatives interact with two conserved His41 and Cys145 residues during whole simulation time in the catalytic sites, suggesting that these derivatives may exert inhibitory effect on SARS COV-2 progression via Mpro inhibition. Taken together, the present in silico study concluded that Shikonin derivatives might play an influential role against Mpro inhibition.

Keywords: SARS COV-2, Main protease, Shikonin derivatives, Molecular docking, Molecular dynamics simulation

P-12-010

Vasorelaxant effect of Trachelospermi caulis extract on rat mesenteric resistance arteries[Chae eun Haam](#), Seonhee Byeon, Sooyeon Choi, Eun Yi Oh, Soo-Kyung Choi, Young-Ho Lee

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Background: Trachelospermi caulis (T. caulis) has been used as a traditional herbal medicine in Asian countries. Although it is well known that T. caulis has beneficial effects, no sufficient research data are available on the cardiovascular effect of T. caulis. We investigated whether T. caulis extract has vascular effects in rat resistance arteries in this study.

Methods: To examine whether T. caulis extract affects vascular reactivity, we measured isometric tension of rat mesenteric resistance arteries using a multi-wire myograph system. T. caulis extract was administered after arteries were pre-contracted with high K⁺ (70 mM) or phenylephrine (5 μ M). Vanillin, a single active component of T. caulis, was used to treat mesenteric arteries.

Results: T. caulis extract caused vascular relaxation in a concentration-dependent manner, which was endothelium-independent. To further identify the mechanism, we incubated the arteries in Ca²⁺-free solution containing high K⁺, followed by a cumulative administration of CaCl₂ (0.01-2.0 mM) with or without T. caulis extract (250 μ g/mL). The treatment of T. caulis extract decreased contractile responses induced by the addition of Ca²⁺, which suggested that the extracellular Ca²⁺ influx was inhibited by the T. caulis extract. Moreover, an active compound of T. caulis extract, vanillin, also induced vasodilation in mesenteric resistance arteries.

Conclusion: T. caulis extract and its active compound, vanillin, concentration-dependently induced vascular relaxation in mesenteric resistance arteries. These results suggest that the administration of T. caulis extract could help decrease blood pressure.

Keywords: Ca²⁺, Trachelospermi caulis, Mesenteric resistance arteries, Re-

laxation, Vanillin

P-12-011

Vascular relaxation induced by vanillin in rat mesenteric resistance arteries[Sooyeon Choi](#), Chae Eun Haam, Eun Yi Oh, Seonhee Byeon, Soo-Kyung Choi, Young-Ho Lee

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Background: Vanillin (4-hydroxy-3-methoxybenzaldehyde, C₈H₈O₃) is a phenolic aldehyde, which is found in plant species of Vanilla genus. Although recent studies have suggested that vanillin has various beneficial properties, the effect of vanillin on blood vessels has not been studied well. In the present study, we investigated whether vanillin has vascular effects in rat mesenteric resistance arteries.

Methods: To examine vascular effect of vanillin, we measured isometric tension of rat mesenteric resistance arteries using a multi-wire myograph system. After arteries were pre-contracted with high K⁺ (70 mM) or phenylephrine (5 μ M), vanillin was administered.

Results: Vanillin induced concentration-dependent vasodilation in rat mesenteric resistance arteries. Endothelial denudation or treatment of endothelial nitric oxide synthase inhibitor (L-NG-nitro arginine, 300 μ M) did not affect the vasodilation induced by vanillin. Treatment of K⁺ channel inhibitor (Tetraethylammonium, 2 mM) or soluble guanylyl cyclase inhibitor (1H-1,2,4 oxadiazolo 4,3-a quinoxalin-1-One, 10 μ M) or cyclooxygenase-2 inhibitor (indomethacin, 10 μ M) did not affect the vanillin-induced vasodilation either. To further identify the mechanism, arteries were incubated with Ca²⁺-free solution containing high K⁺, followed by a cumulative administration of CaCl₂ (0.01-2.0 mM) with or without vanillin (3 mM). The treatment of vanillin decreased contractile responses induced by Ca²⁺ addition.

Conclusion: Vanillin induced concentration-dependent vascular relaxation in rat mesenteric resistance arteries, which was endothelium-independent. Inhibition of extracellular Ca²⁺ influx was involved in vanillin-induced vasodilation. These results suggest the possibility of vanillin as a treatment for hypertension.

Keywords: Ca²⁺, Mesenteric resistance arteries, Relaxation, Vanillin, Vasodilation

P-12-012

Inhibition of lactate dehydrogenase A upregulates mitochondrial proteins and fatty acid oxidation in mouse brown adipocytesAye Hsu Lae^{1,2}, [Soo Kyung Lee](#)^{1,2}, Dat Da Ly^{1,2}, Jaetaek Kim³, Chanbae Park⁴, Kyu-Sang Park^{1,2}¹Department of physiology Yonsei University Wonju College of Medicine, Wonju,²Mitohormesis Research Center Yonsei University Wonju College of Medicine, Wonju,³Division of Endocrinology and Metabolism, Department of Internal Medicine College of Medicine, Chung Ang University, Seoul, ⁴Department of Physiology, Department of Biomedical Sciences Ajou University, Suwon

Lactate dehydrogenase (LDH), a key regulator of glycolysis, is an oxidoreductase enzyme catalyzing the reversible conversion between pyruvate and lactate, which are important substrates for energy metabolism of brown adipocytes. Although several studies have identified the critical role of LDH-A in cancer metabolism, the role in brown adipocytes has not been clearly described. Here, the metabolic consequences of LDH-A or LDH-B inhibition were investigated in mouse primary isolated or immortalized brown adipocytes using genetic and pharmacological interventions. Genetic suppression of Ldha but not Ldhb, increased the expression of mitochondrial proteins including uncoupling protein 1 (UCP1), mitochondrial Ca²⁺ uniporter (MCU), and electron transport chain complexes in brown adipocytes. Peroxisome proliferator-activated receptor gamma coactivator

1- α , as an upstream of mitochondrial biogenesis, was also upregulated by LDH-A knockdown. Interestingly, intracellular ATP level was significantly attenuated by inhibition of LDH-A, which did not occur by LDH-B suppression. As a result of ATP decline, the activity of AMP-activated kinase (AMPK) was augmented by LDH-A knockdown, which was accompanied by inhibition of lipogenic acetyl-coA carboxylase and activation of lipolytic hormone-sensitive lipase. Consistently, oxygen consumption rate for fatty acid oxidation was augmented by LDH-A inhibition. Sodium oxamate, a pyruvate analog as a competitive inhibitor of LDH-A, reiterated the effects of LDH-A knockdown, including upregulations of mitochondrial proteins including UCP1, ATP depletion, AMPK activation, and stimulated lipolysis. Taken together, LDH-A inhibition could be the novel therapeutic target for obesity and metabolic diseases by improving mitochondrial energy metabolism.

Keywords: BAT, LDHA, Oxamate, UCP1

P-12-013

Finasteride ameliorates neointima hyperplasia in a rat carotid balloon injury model and suppresses primary cultured rat vascular smooth muscle cell proliferation and migration.

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Diabetes, hypertension, and hyperlipidemia are the underlying causes of atherosclerosis, and they can result in vascular endothelial injury. Neointima hyperplasia is a characteristic response of various endothelial injuries. In addition, in the process of angioplasty or stent insertion to treat atherosclerosis, the neointima hyperplasia occurs due to factors such as endothelial injury and thrombus formation. Endothelial injury causes migration and proliferation of vascular smooth muscle cells (VSMC), which is a major factor in the neointima formation and vascular stenosis.

Finasteride is a competitive inhibitor of 5 α -reductase, inhibiting the conversion of testosterone to dihydrotestosterone (DHT). Due to this action, finasteride is used to treat androgen-dependent benign prostatic hypertrophy (BPH) and androgenic alopecia. In BPH, finasteride is known to inhibit the angiogenesis of the prostate and reduce microvascular density. Moreover, testosterone and DHT have been reported to be correlated with the onset of hypertension in various disease animal models, and studies have shown that they are particularly involved in the proliferation and migration of VSMC. However, the action of finasteride in the cardiovascular system and its effect on VSMC are not fully understood.

Therefore, for the first time, we tested the effect of finasteride in neointimal hyperplasia formation in balloon catheter-induced-carotid artery-injured rats. In addition, the proliferation, migration, and cellular response to finasteride were investigated using primary cultured VSMCs separated from rat thoracic aorta. Finasteride ameliorates the formation of neointima in vivo and proliferation and migration of primary VSMCs were attenuated dose-dependently. These results suggest that finasteride can be used as a therapeutic agent for preventing neointima hyperplasia.

Keywords: Finasteride, Vascular smooth muscle, Neointima, Hyperplasia, Migration

P-12-014

Peptides derived from voltage-dependent calcium channel beta subunit decrease arterial blood pressure in rats

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Beta subunits of high voltage-gated Ca²⁺ channels (HGCCs) are essential for optimal channel functions, such as channel gating and activation-inactivation kinetics, and trafficking to membrane. In this study, we report, for the first time, that peptide fragments from the beta subunits have potent blood pressure-reducing effects in anesthetized or non-anesthetized rats. Intravenous administration of 16-mer peptide fragments, derived from the interacting regions in beta1 [cacb1(344-359)], beta2 [cacb2(392-407)], beta3 [cacb3(292-307)] or beta4 [cacb4(333-348)] subunits with the HGCC main alpha subunit, decreased the arterial blood pressure in a dose-dependent manner for 5-8 min in anesthetized rats. A single mutated peptide of cacb1(344-359), cacb1(344-359)K357R, showing relatively consistent and potent effect, was crippled by only two amino acid truncation at N-terminal or C-terminal end. By conjugating palmitic acid through the second amino acid, lysine, with cacb1(344-359)K357R, called K2-palm, we extended the blood pressure-reducing effect up to several hours without losing its potency. This prolonged effect on the arterial blood pressure was also observed in non-anesthetized rats. We expect that this serendipitous finding would be a useful tool for hypertension treatment.

Acknowledgement: This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education (NRF-2018R1D1A1B07047469). Most of peptide sequences listed here were patented in R. O. Korea (Patent # 10-2425548, 2022).

Keywords: Voltage-gated calcium channel, Beta subunit, Peptide, Blood pressure

P-12-015

Dietary habits interact with five genetic variants related to dyslipidemia in Korean middle-aged adults

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Lifestyle factors and genetic variation both have an impact on dyslipidemia. It is crucial for individuals with dyslipidemia to modify their dietary patterns considering lipid levels are in particular closely linked to eating habits. In this study, we discovered single nucleotide polymorphisms (SNPs) related to dyslipidemia and identified eating habits that are closely associated with these SNPs in Korean population.

Genotyping was conducted to determine genotypes of 72,298 people and investigate genotypes for 7,079,946 SNPs from Korean Genome and Epidemiology Study (KoGES). After discovering SNPs with genome wide association study (GWAS), we analyzed the interaction between the SNPs and eating and drinking habits.

Five SNPs were found to be associated with dyslipidemia in the entire cohort: rs117026536(LPL), rs651821(APOA5), rs9804646(APOA5), rs9926440(CETP), rs429358(APOE). The consumption of ramen, pork belly, and pork intestines, which are considered unique among Korean food cultures, is highly related to major SNPs. SNP of LPL gene had a significant correlation with ramen and pork intestines intake(p<0.05). In addition, rs9926440 of CETP was associated with pork belly intake, and rs651821 of APOA5 was associated with pork intestines intake(p<0.05). In addition, consumption of coffee and alcohol were highly related to the development of dyslipidemia. Coffee

intake had significant interaction with all five SNPs ($p < 0.05$). Drinking period and drinking soju which is traditional alcohol were related to rs651821 of APOA5 ($p < 0.05$).

The results indicated a significant relationship between eating patterns and SNPs related to dyslipidemia in the Korean middle-aged adults. Korean adults' distinctive dietary habits are closely related to dyslipidemia, therefore guidelines that take this into account seem necessary.

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Keywords: Genetic variants, Dietary habits, Dyslipidemia, GWAS

P-12-016

Role of lateral hypothalamus-lateral habenula pathway in cocaine-induced psychomotor responses

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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. It has been suggested that lateral hypothalamus (LH) or lateral habenula (LHb) is associated with cocaine-induced drug seeking behaviors. To explore the role of an LH-LHb pathway in cocaine-induced psychomotor responses, we tested whether activation or inhibition of the LH-LHb pathway affects cocaine-induced locomotion. Cocaine-induced locomotor activity and dopamine release was suppressed by an activation of LH with PEPA, an AMPA receptor agonist. When LH was inhibited by microinjection of GABA mixtures prior to cocaine injection, the cocaine effects were enhanced. Furthermore, optogenetic activation of the LH-LHb pathway attenuated the cocaine-induced locomotion, while optogenetic inhibition of the LH-LHb pathway increased it. These findings suggest that the LH-LHb circuit plays a role in the modulation of cocaine-induced psychomotor responses.

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Keywords: Lateral hypothalamus, Lateral habenula, Locomotion, Cocaine

P-12-017

Activation of a hypothalamus-habenula circuit by mechanical stimulation inhibits cocaine addiction-like behaviors

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Background: Mechanoreceptor activation modulates GABA neuron firing and dopamine (DA) release in the mesolimbic DA system, an area implicated in reward and substance abuse. The lateral habenula (LHb), the lateral hypothalamus (LH), and the mesolimbic DA system are not only reciprocally connected, but also involved in drug reward. Objective: We explored the

effects of mechanical stimulation (MS) on cocaine addiction-like behaviors and the role of the LH-LHb circuit in the MS effects.

Methods: MS was performed over ulnar nerve and the effects were evaluated by using drug seeking behaviors, optogenetics, chemogenetics, electrophysiology and immunohistochemistry.

Results: Mechanical stimulation attenuated locomotor activity in a nerve-dependent manner and 50-kHz ultrasonic vocalizations (USVs) and DA release in nucleus accumbens (NAc) following cocaine injection. The MS effects were ablated by electrolytic lesion or optogenetic inhibition of LHb. Optogenetic activation of LHb suppressed cocaine enhanced 50 kHz USVs and locomotion. MS reversed cocaine suppression of neuronal activity of LHb. MS also inhibited cocaine-primed reinstatement of drug-seeking behavior, which was blocked by chemogenetic inhibition of an LH-LHb circuit.

Conclusion: These findings suggest that peripheral mechanical stimulation activates LH-LHb pathways to attenuate cocaine-induced psychomotor responses and seeking behaviors.

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Keywords: Mechanical stimulation (MS), Cocaine, Lateral habenula, Locomotor activity, Ultrasonic vocalization (USVs), Optogenetics

P-13-001

Coffee consumption may promote sudomotor function activation

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The purpose of this study was to determine whether drinking coffee with caffeine accelerates the sympathetic response to acetylcholine (ACh). Tests were performed twice at 1-week intervals following the intake of coffee. Subjects were randomly divided into two groups: Group A was administered 16 fluid oz of water (CON), while Group B was given 16 fluid oz of coffee (Coffee). After 1 week, Group A was administered 16 fluid oz of coffee (Coffee), while Group B was given 16 fluid oz of water (CON). The quantitative sudomotor axon reflex test (QSART) was performed after intake of coffee and water and a 40 min break. QSART with iontophoresis and 10% ACh was performed to determine axon reflex (AXR) mediated with and without iontophoresis [AXR (1) and AXR (2), respectively], and directly activated sweating (DIR). The sweat onset time of the AXR was shorter in the Coffee compared with the CON. The sweat rates in AXR (1) AXR (2) and DIR were significantly higher in the Coffee than in the CON. In addition, the Coffee showed a significantly higher density of activated sweat glands and activated sweat gland output than the CON. The overall results of this study showed that coffee intake could stimulate higher activation in both AXR and DIR sweat responses. In addition, it has been shown that coffee intake can improve sweating sensitivity by the contribution of caffeine contained in coffee. This suggests that other compounds in coffee may not inhibit the sympathetic response to ACh. Therefore, coffee may be clinically worth considering as a supplement for activation of sudomotor function and increase of thermogenesis.

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Keywords: Coffee, Caffeine, QSART, Axon-reflex, Sudomotor function

P-13-002

Effect of exercise intensity on blood irisin, FGF21, adiponectin, DA and 5-HT levels

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To determine effects of exercise intensity on expression levels of cytokines beneficial for the health (especially, improvement of obesity and obesity-related metabolic diseases) and central nervous system (CNS) fatigue. Expression levels of irisin, fibroblast growth factor-21 (FGF21), adiponectin, free fatty acid (FFA), dopamine (DA), and serotonin (5-HT) levels after 50% of maximal oxygen uptake (VO₂max) of treadmill running and 80% VO₂max of treadmill running for 30 min in 20 healthy men were compared. Blood samples were collected at three time points: before treadmill running (pre-EX), immediately after treadmill running (post-EX), and at 60 min after treadmill running (60 min post-EX). Expression levels of irisin, FGF21, adiponectin, FFA, DA, and 5-HT were increased after 30 min of treadmill running exercise regardless of exercise intensity. Their levels were increased at 60 min post-EX. They showed no significant difference immediately after exercise regardless of exercise intensity. Only irisin, FGF21, FFA, and DA levels showed significant differences between 50% VO₂max group and 80% VO₂max group at 60 min post-EX. In conclusion, acute moderate-intensity exercise increases blood levels of irisin and FGF21 to a lesser extent than high-intensity exercise but may delay CNS fatigue in healthy humans.

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Keywords: Irisin, FGF21, Adiponectin, Dopamine, Serotonin

P-13-003

Effects of caffeine ingestion and thermotherapy on blood orexin circulation in humans

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Caffeine and orexin can affect awakening, neuroendocrine, and sympathetic nerve function. Our previous study has reported that caffeine intake can increase human body temperature. This time, we investigated the combined effect of caffeine intake and hyperthermia on blood orexin concentrations in humans compared to thermal stimulation alone. Forty-two healthy male subjects with age of 26.72 ± 5.05 years, height of 174.10 ± 7.09 cm, and body weight of 74.68 ± 8.91 kg participated in this study. They were randomly assigned to a control (CON) group and a caffeine (CAFF) group. After thermotherapy (half-body immersion in a hot water bath at $42 \pm 0.5^\circ\text{C}$, circulating orexin level increased more ($p < 0.05$) in the CAFF group than in the CON group. After thermotherapy, circulating orexin level increased more with caffeine consumption, and positive relationships between mean body temperature and orexin were observed before and after heat stimulation. Our results suggest that caffeine boosted the upregulation of serum orexin concentrations in subjects undergoing thermotherapy via sympathetic nervous system, and could be used as a novel form of therapy with potential to treat neurological and cardiovascular diseases.

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Keywords: Caffeine, Orexin, Thermotherapy, Neuroendocrine, Sympathetic nervous system

P-13-004

Psychological and Physiological Effects of Dance Movement Therapy on Depression of Juvenile Adolescents through Cortisol and Serotonin

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The purpose of this study was to analyze the psychophysiological differences between delinquent adolescents and general school adolescents before and after participating in dance movement therapy (DMT). The participants in this study consisted of a total of 42 students of a juvenile training school: 26 female students with an average age of 16 years old and 16 female adolescents with an average age of 16 years old in juvenile training school. For psychological testing, the Beck Depression Inventory (BDI) scale was taken before and after DMT, and for physiological indicators, a blood test was taken to analyze serotonin (5-HT), cortisol. After 12 weeks of dance movement therapy, the delinquent adolescents showed significantly reduced scores on depression scale BDI, and there were statistically significant differences in cortisol, 5-HT between delinquent adolescents and general adolescents. Although it is difficult to generalize the results of this study, DMT was found to be effective in lowering the degree of depression in delinquent adolescents according to BDI, and it was also shown to significantly alter cortisol, 5-HT.

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Keywords: Dance movement therapy, Juvenile Delinquents, Depression, Cortisol, Serotonin (5-HT)

P-13-005

Heat acclimation affects circulating levels of irisin, orexin and COX-2 in humans

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We examined serum levels of irisin, orexin and cyclooxygenase (COX)-2 before and after heat acclimation (HA) to test the hypothesis that decreased body temperature due to HA reduces circulating levels of these key thermoregulatory molecules. Sixteen healthy human male volunteers were recruited (age, 24.8 ± 2.6 years). The subjects were exposed to half-body immersion in hot water ($42 \pm 0.5^\circ\text{C}$) at the same time of day (2–5 pm) on alternate days for 4 weeks. The HA protocol included 10 bouts of 30 min immersion. All experiments were performed in an automated climate chamber (temperature, $26.0 \pm 0.5^\circ\text{C}$; relative humidity, $60 \pm 3.0\%$; air velocity, < 1

m/sec). Tympanic and skin temperatures were measured, and mean body temperatures were calculated. The difference in body weight was used to estimate total sweat loss. Blood levels of irisin, orexin and COX-2 were analyzed before and after HA. Body temperature decreased significantly ($P < 0.05$) after HA, whereas sweat volume increased significantly ($P < 0.01$). Blood irisin, orexin and COX-2 concentrations decreased significantly compared to those at pre-acclimation ($P < 0.01$, $P < 0.01$, $P < 0.01$, respectively). Our data suggest that decreased basal body temperature after HA was associated with decreases in fever-related molecules, such as irisin and COX-2, and the thermogenesis-related molecule orexin.

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Keywords: Heat-acclimatization, Irisin, Orexin, Cyclooxygenase, Body temperature

P-13-006

Sudomotor function evaluated by quantitative direct and axon reflex test in human

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The aim of this study was to quantitatively investigate peripheral sudomotor function through axon reflex-mediated (AXR) and directly activated (DIR) sweating in healthy male and female subjects in the 20's-70's groups and to evaluate the correlation between age and sweating function. Quantitative sudomotor axon reflex testing (QSART) with iontophoresis (2 mA for 5 min) and 10% acetylcholine (ACh) were performed to determine AXR and DIR sweating. All experiments were conducted in a thermoneutral condition (temperature, $24.0 \pm 0.5^\circ\text{C}$; relative humidity, $40 \pm 3\%$). The onset time of AXR ($P < 0.01$) was positively correlated with advancing age, whereas sweat rates of AXR (1) (with ACh 10% iontophoresis) and AXR (2) (without iontophoresis) (respectively, $P < 0.01$), DIR ($P < 0.01$), activated sweat gland density ($P < 0.01$) and sweat gland output ($P < 0.01$) were negatively correlated in the two genders with advancing age. Differences between males and females were observed in all age groups. These observations suggest that an attenuation of sudomotor function occurs with ageing due to neurodegeneration resulting from nerve fiber demyelination. Variation in sweating between sexes exists in all age groups.

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Keywords: Acetylcholine (ACh), Sweating, Activated sweat gland density, Activated sweat gland output, QSART

P-13-007

Effects of music therapy as an alternative treatment on depression in children and adolescents with ADHD

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The objective of this study was conducted 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. A total of 43 subjects participated in the experiment, consisting of an ADHD group ($n = 18$, age of 12.15 ± 2.43 years; height of 142.57 ± 9.98 cm; weight of 41.45 ± 13.87 kg) and a control group ($n = 25$, with age, height, and weight similar to those with ADHD).

Music therapy was regularly performed for all subjects twice a week for three months, 50 minutes each, for a total of 24 times. From a neurophysiological perspective, changes in depression and stress were tracked by measuring 5-HT secretion, cortisol expression, and psychological scales. After music therapy was applied, as a result of the measurement, in both ADHD group and control group, 5-HT secretion had increased whereas cortisol expression. The CDI, DHQ psychological scale also showed positive changes ($p < 0.05$).

Keywords: Children and youth with ADHD, Music therapy, Serotonin (5-HT), Depression, Cortisol

P-13-008

Thermotherapy as an alternative to exercise for metabolic health in obese postmenopausal women: Focus on circulating irisin level

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Irisin is a myokine caused by exercise that improves insulin resistance and weight loss. However, under unfavorable conditions such as air pollution, and during the epidemic, outdoor activities uncomfortable. Therefore, in this study, the effect of heat therapy (half bath $42 \pm 0.5^\circ\text{C}$ for 30 min) on irisin circulation levels as an exercise alternative for middle-aged obese women after menopause was investigated. Subjects were 33 women aged 49.54 ± 6.04 years, with parameters of height, 160.12 ± 4.33 cm, weight, 69.71 ± 7.52 kg, body surface area 1.73 ± 0.13 m², body mass index, 27.19 ± 3.40 kg/m². The results suggest that circulating irisin levels showed a significant increase after one-time thermotherapy (TH-1). However, the increase in circulating irisin levels after 15 treatments (TH-15, 5 days/week, 3 weeks) was significantly varied. The level of adiponectin, which increases fatty oxidation to reduce fatty deposition, increased significantly at TH-1, but further increased at TH-15, which was significantly different from the level of TH-1. In addition, the basic serum free fatty acid (FFA) level was significantly increased at TH-15 compared to TH-1. Significant differences were also found in the lipid profile (body mass index, waist circumference, and % body fat). Thermotherapy can significantly increase the tympanic temperature and induce changes in circulating irisin and adiponectin levels. Thus, it resulted in positive changes in FFA and lipid profiles. Therefore, repeated thermotherapy is effective in increasing circulating irisin levels in postmenopausal

obese women.

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Keywords: Thermotherapy, Half bath, Irisin, Adiponectin, FFA

P-13-010

Firefighters' thermal and immune-inflammatory responses in a hot and humid environment

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Although firefighters' thermal burden during hot and humid summer are one of the most-frequently raised research topics, there were relatively few studies on relationships between immune or inflammatory responses and physiological burden of firefighters. This study aimed to explore relationships between thermal physiological burdens and immune-inflammatory responses of firefighters who wear personal protective equipment in a hot and humid environment. Twenty-eight firefighters (37.5 ± 9.6 y in age, 175.6 ± 5.5 cm in height, 79.9 ± 11.2 kg in weight, and 25.4 ± 2.9 kg·m⁻² in BMI) participated in a PPE conditions (undershorts, station uniform [shirts and pants], socks, turnout jacket and pants, protective hood, helmet, boots and self-contained breathing apparatus [SCBA], total 17.2 ± 0.2 kg in PPE mass). Subjects repeated twice a bout of exercise and recovery (30-min exercise at 5.0 km·h⁻¹ on a treadmill, and 20-min recovery) along with the initial rest of 10 min (REST). During the recovery, subjects took off their helmet, hood, gloves, turnout jacket, and SCBA. All trials were conducted in a climate chamber with a temperature of $30.1 \pm 0.4^\circ\text{C}$ and humidity of $61 \pm 1\%$ RH. Blood sampling were carried out three times on the forearm: prior to entering the climate chamber (PRE), the first recovery (RCV1), and the second recovery (RCV2). The rectal temperature was continuously measured every 5 s. A one-way repeated-measure ANOVA and Pearson's correlation analysis were undertaken, and the statistical significance level was set at 0.05. The results showed that significant differences were found in rectal temperature among the phases ($37.0 \pm 0.2^\circ\text{C}$ for REST < $37.8 \pm 0.3^\circ\text{C}$ for RCV1 < $38.7 \pm 0.5^\circ\text{C}$ for RCV2, $P < 0.001$). Blood platelet, white blood cells, and IL-6 were significantly greater during the RCV2, than PRE ($P < 0.001$). TNF- α and C-reactive protein (CRP) showed significant difference among the phases (PRE \leq RCV1 < RCV2, $P < 0.001$). We confirmed that the immune-inflammatory responses, in terms of blood platelet ($r = 0.525$, $P < 0.001$), white blood cells ($r = 0.402$, $P < 0.001$), IL-6 ($r = 0.341$, $P = 0.003$) showed linear increases as core body temperature increased while thermal burden was intensified.

Keywords: Firefighter, Personal protective equipment (PPE), Immune response, Inflammatory response, Cardiovascular burden

as a thermal stimulator. Thermal thresholds using the radiant film heater were measured on the forehead, abdomen, forearm and foot at an air temperature of $22.6 \pm 0.5^\circ\text{C}$ with $48 \pm 12\%$ RH. The surface temperature of the radiant heat panel was maintained at 200°C and the distance from the skin to the heating panel was kept at 10 cm during the measurements. Skin temperatures (Tsk) on the body regions were monitored every 1 s. A thermal threshold was defined as Tsk of the moment of that subjects initially felt warm, hot or pain sensation on the skin. Warmth thresholds showed no significant differences between the two groups ($35.4 \pm 1.1^\circ\text{C}$ and $35.2 \pm 1.4^\circ\text{C}$ on the forehead for the elderly and young, respectively; $34.2 \pm 1.5^\circ\text{C}$ and $32.8 \pm 0.9^\circ\text{C}$ on the forearm; $34.7 \pm 1.5^\circ\text{C}$ and $34.0 \pm 1.0^\circ\text{C}$ for the abdomen; $33.9 \pm 2.9^\circ\text{C}$ and $33.1 \pm 2.4^\circ\text{C}$ on the foot). There were no age differences in hot sensation thresholds ($36.8 \pm 1.7^\circ\text{C}$ and $37.1 \pm 1.4^\circ\text{C}$ on the forehead for the elderly and young, respectively; $36.4 \pm 2.0^\circ\text{C}$ and $35.3 \pm 1.8^\circ\text{C}$ on the forearm; $37.0 \pm 1.9^\circ\text{C}$ and $37.0 \pm 2.0^\circ\text{C}$ on the abdomen; $35.8 \pm 2.8^\circ\text{C}$ and $37.0 \pm 1.9^\circ\text{C}$ on the foot). Heat pain thresholds did not show any significant differences between the two groups ($38.1 \pm 1.9^\circ\text{C}$ and $38.9 \pm 1.9^\circ\text{C}$ on the forehead; $37.7 \pm 1.9^\circ\text{C}$ and $37.2 \pm 2.1^\circ\text{C}$ on the forearm; $38.5 \pm 2.1^\circ\text{C}$ and $39.3 \pm 2.5^\circ\text{C}$ on the abdomen; $37.0 \pm 2.9^\circ\text{C}$ and $38.5 \pm 1.8^\circ\text{C}$ on the foot). However, body regional differences in thermal thresholds were significant for the both elderly and young ($P < 0.05$), which indicates that the foot was more insensitive to detect cutaneous radiant heat on the skin when compared to the forehead or forearm. The baseline Tsk was significantly lower on the foot than other body regions, while the increases in Tsk to detect warmth or hotness sensation were greater on the foot than the forehead or forearm. In summary, age-related differences in thermal thresholds during the radiant heat exposure were not marked, but body regional differences were noticeable for the both young and older groups.

Keywords: Ageing, Thermal sensitivity, Thermal threshold, Body region

P-13-011

Age-related differences in cutaneous thermal thresholds on the trunk and periphery

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The purpose of the present study was to investigate age-related differences in cutaneous warm, hot and pain sensitivity on the trunk and periphery. Fifteen young males (23.9 ± 2.4 y, 172.7 ± 5.6 cm in height, 75.4 ± 16.3 kg in body weight, 25.2 ± 4.6 kg/m² in BMI) and 15 older males (74.7 ± 4.2 y, 162.8 ± 6.9 cm in height, 64.8 ± 10.4 kg in body weight, 24.3 ± 2.8 kg/m² in BMI) participated in this study. A radiant heat panel (20 x 20 cm) was used