**1-B-YIA1-1** 一般演題（YIA）

# Caveolin-1 modulates P2X7 receptor-dependent ATP signaling in pro-inflammatory macrophages.

Yuuki Sawai, Yoshiaki Suzuki, Rubii Kondo, Yuji Imaizumi, Hisao Yamamura

*Dept. Mol & Cell. Pharmacol., Grad. Sch. Pharmaceut. Sci., Nagoya City Univ.*

[Background] Macrophage（M$\phi$）is one of the innate immune cells and related to several chronic inflammatory diseases. Ionotropic purinergic P2X7 receptor plays a key role in the regulation of M$\phi$ functions. Caveolin-1（Cav-1）is known to modulate the activities of ion channels. In this study, the functional coupling between Cav-1 and P2X7 receptor was examined using Cav-1 knockout（Cav-1 KO）mice.
[Methods] Murine bone marrow-derived M$\phi$（BMDM）was used in this study. Molecular interaction between Cav-1 and P2X7 receptor was analyzed by proximal ligation assay（PLA）. Intracellular [$Ca^{2+}$] and [$K^{+}$] were measured by confocal microscopy with each specific fluorescent indicator. Lytic cell death was analyzed by LDH assay.
[Results] In BMDMs, Cav-1 expression was increased by lipopolysaccharide. PLA revealed the molecular interaction between Cav-1 and P2X7 receptor. The treatment with 1 mM ATP evoked sustained $Ca^{2+}$ influx and $K^{+}$ efflux in BMDMs from wild-type mice. The response was enhanced in BMDMs from Cav-1 KO mice. ATP stimulation induced lytic cell death through P2X7 receptor activation, and this cell death was facilitated in Cav-1 KO mice.
[Conclusion] Cav-1 negatively regulates the activation of P2X7 receptors and results in cell death in M$\phi$. This study may lead to the development of novel drugs of chronic inflammatory diseases.

**1-B-YIA1-2** 一般演題（YIA）

# Possible role of food additives on formation of leptin resistance in obesity

Shibuya Yuki[1], Kyoshiro Tsuge[2], Karen Kuriya[1], Tadashi Nakagawa[1], Ralf Jockers[3], Julie Dam[3], Akira Shimamoto[2], Toru Hosoi[1]

*[1]Dept. Clin. Pharmacology, Facul. Pharmaceutical Sci., Sanyo-Onoda City Univ., [2]Dept. Regenerative Medicine Research, Facul. Pharmaceutical Sci., Sanyo-Onoda City Univ., [3]Inserm U1016, Institut Cochin, Dept Endocrinology, Metabolism and Diabetes, Paris, France*

Obesity is known to be triggered by various factors and is a recognized as a risk factor for metabolic diseases. Leptin is an anti-obesity hormone, which can inhibit food intake and increase energy expenditure. It is suggested that leptin resistance is one of the underlying mechanisms of obesity. Our research group is studying the impact of endoplasmic reticulum (ER) stress on development of leptin resistance. We previously found that 4-hydroxynonenal, which contains aldehyde, induced ER stress and inhibited leptin-induced STAT3 phosphorylation. In the present study, we identified several food additives, which contains aldehydes that could potentially cause leptin resistance through screening using STAT3 luciferase assay at neuronal cells expressing Ob-Rb leptin receptor. We also evaluated the downstream of leptin signal by analyzing STAT3 signal by Western blotting. We found that the several food additive can inhibit leptin-induced STAT3 signal. We are now analyzing the effect of food additives on ObR leptin receptor function by BRET technique. Overall, our results suggests that some food additives, which contain aldehydes, may potentially lead to obesity through formation of leptin resistance.

**1-B-YIA1-3** 一般演題（YIA）

# Identification of a novel circulating factor that denervate neuromuscular junction in Amyotrophic lateral sclerosis (ALS) model mouse

Lili Quan, Shogo Tanabe, Rieko Muramatsu

*Dept. Mol. Pharmacol., Natl. Inst. Neurosci., Natl. Center Neurol. Psychiat.*

Amyotrophic lateral sclerosis (ALS) is a rare but devastating disease characterized by progressive neurodegeneration of motor neurons, leading to skeletal muscle denervation. Neuromuscular junction (NMJ) degeneration is an early pathological change in ALS. Nonetheless, the exact regulatory mechanisms and potential therapeutic interventions underlying this process remain largely unknown. Through our preliminary experiment using heterochronic parabiosis, a surgical technique enabling blood circulation sharing between organisms, we coupled ALS mice ($SOD1^{G93A}$) with WT mice. Surprisingly, this led to NMJ shedding in the WT mice, a phenomenon not typically observed, thus hinting at the presence of NMJ-degenerating molecules in ALS mouse plasma. Next, we conducted an extensive analysis of cytokine levels in the plasma of $SOD1^{G93A}$ mice and WT mice and found markedly elevated levels of cytokine X in the ALS mice plasma, with abundant production originating from intestinal vascular endothelial cells. Treating $SOD1^{G93A}$ mice with neutralizing cytokine X antibodies significantly suppressed NMJ degeneration and improved sciatic compound muscle action potential (CMAP). Similarly, a comparable positive outcome was also observed with the treatment of lipid nanoparticles containing cytokine X receptor siRNA formulations in the skeletal muscle tissue of $SOD1^{G93A}$ mice. In addition to exploring the correlation between nervous system cell dysfunction within the lesion, our study unveiled a crucial role of systemically expressed cytokine X in ALS pathogenesis, offering valuable insights into a potential therapeutic biomarker for the disease.

**1-B-YIA1-4** 一般演題（YIA）

# $NAD^+$ level at a young age affects skeletal muscle functions at an old age

Mariam Karim, Takashi Nakagawa

*Dept. Mol. Med. Pharmacol., Toyama Univ.*

Nicotinamide adenine dinucleotide ($NAD^+$) is an essential coenzyme involved in many cellular functions including energetics, circadian rhythm, transcription, and epigenetics etc. Imbalanced $NAD^+$ levels lead to age-related pathologies. $NAD^+$ is biosynthesized by *de novo*, Preiss-Handler, and salvage pathways. It has been shown that the salvage pathway is more vital in maintaining $NAD^+$ levels in skeletal muscle. Whereas evidence proving the role of *de novo* and Preiss-Handler pathways remains elusive. NAD synthetase (NADS) is the last enzyme shared in *de novo* and Preiss-Handler pathways. In this study, we generated NADS KO mice and investigated the role of *de novo* and Preiss-Handler pathways in skeletal muscle. The skeletal muscle in NADS KO mice showed depleted $NAD^+$ levels at a young age resulting in poor skeletal muscle performance assessed by grip strength and endurance on the treadmill. Histology of these mice showed decreased cross-sectional area resembling sarcopenia. Interestingly, old NADS KO mice restored $NAD^+$ levels but these mice didn‘t recover muscle strength and endurance suggesting that reduced $NAD^+$ levels in young age influence exercise performance in old age. Further, RNA sequencing of skeletal muscle revealed circadian genes are altered in NADS KO mice. In addition, we found that NAD+ precursor replenishment rescued the $NAD^+$ levels and muscle weakness in NADS KO mice. This study implies that maintaining $NAD^+$ levels in young age is crucial in preventing skeletal muscle aging.

1-B-YIA1-5 一般演題（YIA）

# Insulin secretory and anti-apoptotic effect of apigenin on INS-ID pancreatic β-cell

Ihim Stella, Yukiko Kaneko, Moe Yamamoto, Tomohisa Ishikawa

*Dept. Pharmacol., Sch. Pharm. Sci., Univ. Shizuoka*

The vulnerability of pancreatic β-cells to endoplasmic reticulum stress (ER), is a common cause of β-cell apoptosis and dysfunction involving decreased insulin secretory response and a reduction in β-cell mass in type 2 diabetes mellitus. The possible role of naturally occurring polyphenols –known as flavonoids- in treating type 2 diabetes mellitus is a current area of focus. However, there is a dearth of information about the effect of the trihydroxyflavone, apigenin, on pancreatic β-cell functions.

We evaluated the effect of apigenin on glucose-induced insulin secretion (GSIS) and β-cell apoptosis, studying the mechanism underlying its antidiabetic effects, using the INS-ID β-cell line. The results showed that apigenin dose-dependently stimulated GSIS at all concentrations (1, 10, 30 and 100 μM), with a significant peak effect at 30 μM concentration. The downstream ER stress signaling proteins, CHOP and cleaved caspase-3, which were elevated by thapsigargin-induced apoptosis of INS-1 cells, were concentration-dependently and strongly attenuated by apigenin treatment at all concentrations, with peak suppression at concentrations of 30 and 100 μM. These results strongly correlated with the flow cytometric analysis of Annexin V/PI staining and the DNA fragmentation analysis, which is indicative of apoptosis, as determined by DNA laddering. The thapsigargin-induced increase in TXNIP expression was also dose-dependently suppressed by treatment with apigenin. Apigenin also inhibited high concentration of glucose-induced apoptotic increases in cleaved caspase-3 and CHOP expressions.

These results suggest that apigenin is an attractive candidate with remarkable and potent insulinotropic and anti-apoptotic effects on β-cells and that its anti-diabetic effect may be mediated by increasing GSIS and preventing ER stress-mediated β-cell apoptosis mediated by CHOP, cleaved caspase-3 and TXNIP, hence promoting β-cells survival and function.

1-B-YIA2-1 一般演題（YIA）

# Crosstalk among the skeletal muscle, liver and adipose tissue on glycemic control via peripheral sympathetic nervous system in rats and its attenuation with high-fat diet

Daisuke Sato[1], Nozomi Imaizumi[1], Sae Itabashi[2], Ryoichi Banno[3,4], Masataka Kusunoki[3], Licht Miyamoto[5]

*[1]Dept. Biochem. Eng. Yamagata Univ., [2]Dept. Biochem. Eng. Yamagata Univ., [3]Res. Ctr. Health Phys. Fitness Sports Nagoya Univ., [4]Dept. Diabetes Endocrinol. Nagoya Univ. Hosp., [5]Dept. Nutr. Life Sci. Kanagawa Inst. Tech.*

We previously reported that peripheral sympathetic nervous activation enhanced glucose uptake in standard laboratory chow- (SCF) and high-fat-fed (HFF) rats, and that the uptake might be mediated by PGC-1$\alpha$ in the soleus muscle in the SCF rats. However, blood glucose (BG) reduction was only temporal. In the present study, we investigated the effects of peripheral sympathetic activation on glycemic control. We detected sympathetic signal with a microelectrode in the unilateral sciatic nerve under anesthetic condition in the SCF and HFF rats, and conducted electrical microstimulation (MS) via the electrode for 60 min. The MS resulted in no significant change or slight elevation of BG in the SCF or HFF rats, respectively without no change in plasma insulin level. In the SCF rats, the MS elevated *G6Pase* mRNA expression as well as glycogen content in the liver, and reduced triglyceride (TG) content in the white adipose tissue. Conversely, the MS had no effects on these parameters in the HFF rats. These results suggest that peripheral sympathetic activation enhances glucose uptake independently of insulin in the skeletal muscle while BG level might be compensated by hepatic glucose production, and that the TG reduction in white adipose tissue may lead to glycerol supply and gluconeogenesis in the liver in the SCF but not in HFF rats.

1-B-YIA2-2 一般演題（YIA）

# Amitriptyline potentiates the expression of brain-derived neurotrophic factor by enhancing downstream signal of lysophosphatidic receptors in connexin43 knockdown astrocyte

Nozomi Tokunaga, Yoki Nakamura, Kazue Hisaoka-Nakashima, Norimitsu Morioka

*Dept. Pharmacol., Grad. Sch. Biomed. Health Sci., Hiroshima Univ.*

Connexin43 (Cx43) is highly expressed in astrocytes (AS), and its expression is reduced in the prefrontal cortex of depressive patients. We have previously demonstrated amitriptyline (AMI), a tricyclic antidepressant, increased the expression of brain-derived neurotrophic factor (BDNF) mediated by lysophosphatidic acid (LPA) receptors. Present study examined the detailed mechanism involved in these responses. Primary cultured AS were prepared from the cerebral cortex of neonatal Wistar rats. The expression of Cx43 was downregulated with RNA interference. The expression of mRNA and protein were measured by real-time PCR and western blotting, respectively. Protein kinase C (PKC) activity was measured by using the assay kit. The enhancement of BDNF expression in Cx43-knockdown (KD) AS was suppressed by inhibition of extracellular signal-regulated kinase (ERK) and PKC which were known as the downstream of LPA receptors. Moreover, ERK phosphorylation and PKC activity were increased by treatment with AMI, which were also enhanced in Cx43-KD AS. Present study revealed reduced Cx43 expression potentiated the AMI-induced BDNF expression by enhancement of intracellular signaling in cortical AS. These results indicate the downregulation of Cx43 observed in the depressive patients might contribute to therapeutic effect of antidepressants.

1-B-YIA2-3 一般演題（YIA）

# Synchronous firing promotes the formation of synaptic connectivity contributes to the brain function

Kashima Tetsuhiko[1], Takashi Yoshida[2,3,4], Kenichi Ohki[2,3,4], Yuji Ikegaya[1,2]

*[1]Grad Sch. Pharma Sci, Univ. Tokyo, Tokyo, Japan,[2]Inst. for AI and Beyond, Univ. Tokyo, Tokyo, Japan,[3]Grad Sch. Medicine, Univ. Tokyo, Tokyo, Japan,[4]WPI-IRCN, The University of Tokyo, Japan*

The proper development of neural circuits is essential for normal brain function and is believed to be governed by the Hebbian rule a well-known hypothesis in neural circuit research, which suggests that neurons that fire together wire together. However, despite its long-standing history as a hypothesis, direct evidence supporting this notion and its influence on brain function has remained elusive. Our research investigated the Hebbian rule by experimentally inducing synchronous firing during the development of neural circuits in the mouse visual cortex. This brain region is known for its close relationship between neural circuit structure and brain function. To achieve this, we utilized a non-invasive transcranial optogenetic stimulation method, which allowed us to induce synchronous firing in ChR2-positive neurons during a critical period. We revealed a higher connection probability between synchronized neurons compared to ChR2-negative neurons or the control group without photostimulation, and this increase in connection probability was prevented by chronic treatment with MK801, an NMDAR antagonist. We used two-photon calcium imaging to examine the orientation selectivity of the stimulated neurons and found that synchronized neurons responded more similarly than the others. The increases in synaptic connectivity and similarity of orientation selectivity were not observed in randomly fired conditions. These outcomes suggest that developmental synaptic connection and its function depend not on the firing rate but on synchronicity.

1-B-YIA2-4 一般演題（YIA）

# Investigating the effects of neurotrophin-3 overexpression on the hippocampal dentate gyrus

Kasakura Nanami[1], Yuka Murata[1], Asuka Shindo[1], Shiho Kitaoka[2], Tomoyuki Furuyashiki[3], Kanzo Suzuki[1], Eri Segi-Nishida[1]

*[1]Dep. of Bio. Sci. and Tech., Tokyo Univ. of Sci.,[2]Dep. of Pharm., Hyogo Med. Univ., [3]Div. Pharmacol., Grad. Sch. Med., Kobe Univ*

Neurotrophin-3 (NT-3) is a type of neurotrophic factor and regulates neural differentiation, survival, and plasticity in both peripheral and central nervous systems. NT-3 expresses in the adult hippocampal dentate gyrus (DG) and has been reported to be upregulated by stress. However, the detailed function of NT3 in the hippocampal DG is unknown. Therefore, we tried to clarify the function of NT3 by overexpressing NT-3 in the hippocampal DG. We generated NT-3 overexpressing mice in the hippocampus by administering adeno-associated virus carrying NT-3 gene. Expression of NT-3 mRNA in the hippocampus was higher more than 35-fold than in the control group. The expression of calbindin, a mature neuronal marker, and the number of FosB positive cells were increased in mature neurons by NT3 overexpression. Moreover, the number of proliferating cells and immature neurons in the hippocampal dentate gyrus was decreased by NT-3 overexpression. In addition, the expression of Vegfd, a factor regulating neurogenesis, was decreased. These results showed that high NT-3 levels in the hippocampus regulate the activation of mature DG neurons and suppress the early phase of neurogenic processes.

1-B-YIA2-5 一般演題（YIA）

# Selective Rho-kinase 2 inhibitor ameliorates the decreased spine density in the medial prefrontal cortex of mice carrying the variants of *Arhgap10* gene found in a Japanese schizophrenia patient

Rinako Tanaka[1], Wenjun Zhu[1], Daisuke Mori[2], Akihiro Mouri[3], Taku Nagai[4], Toshitaka Nabeshima[5], Kozo Kaibuchi[6], Daiki Tachibana[7], Yohei Kobayashi[7], Norio Ozaki[2], Hiroyuki Mizoguchi[1], Kiyofumi Yamada[1]

*[1]Dept. of Neuropsychopharmacol. & Hosp. Pharm., Nagoya Univ. Grad. Sch. of Med., Nagoya, Japan, [2]Pathophysiol. of mental disorders, Nagoya Univ. Grad. Sch. of Med., Nagoya, Japan, [3]Dept. of Regulatory Sci. for Evaluation & Development of Pharmaceut. & Devices, Fujita Health Univ. Grad. Sch. of Health Sci., Toyoake, Japan, [4]Div. of Behavioral Neuropharmacol., International Ctr. for Brain Sci. (ICBS), Fujita Health Univ., Toyoake, Japan, [5]Lab. of Health & Med. Sci. Innov., Fujita Health Univ. Grad. Sch. of Health Sci., Toyoake, Japan, [6]Div. of Cell Biol., ICBS, Fujita Health Univ., Toyoake, Japan, [7]Pharmacol. Res. Unit, Sumitomo Pharma Co., Ltd., Osaka, Japan*

Copy number variants in the *ARHGAP10* gene are associated with schizophrenia (SCZ). We have previously demonstrated that Rho-kinase (ROCK) inhibitor, fasudil, ameliorates the decreased spine density in the medial prefrontal cortex (mPFC) of *Arhgap10* S490P/NHEJ mice carrying the variants that mimic the *ARHGAP10* variants found in a Japanese SCZ patient. Accordingly, we have proposed that ROCK is a potentially novel therapeutic target in SCZ. It is well known that there are two subtypes of ROCK, ROCK1 and ROCK2, and that fasudil inhibits both subtypes. Since ROCK2 is highly expressed in the brain, here we evaluated the effect of a selective ROCK2 inhibitor, belumosudil (KD025), on spine density in *Arhgap10 S490P/NHEJ* mice. We measured the spine density of pyramidal neurons in layer 2/3 of the mPFC in *Arhgap10* S490P/NHEJ mice following daily oral administration of KD025 for one week. Moreover, we evaluated the general behaviors in an open field and systolic blood pressure after KD025 treatment. KD025 ameliorated decreased spine density of cortical neurons in the mPFC of *Arhgap10* S490P/NHEJ mice, but had little effects on general behaviors and systolic blood pressure induced by fasudil. These observations suggest that ROCK2 is a more appropriate therapeutic target in SCZ, with little inducibility of hypotension.

1-B-YIA3-1 一般演題（YIA）

# Evaluation of approach and avoidance behavior in a three-compartment conflict task in mice.

Natsuko Hitora-Imamura[1,2], Yuki Honshuku[1,2], Yurika Miyagami[2], Aoi Mori[2], Hiroshi Nomura[2,3], Masabumi Minami[2]

*[1]Fac. Life Sci., Kumamoto Univ., [2]Dept. of Pharmacol., Grad. Sch. of Pharmaceut. Sci., Hokkaido Univ., [3]Dept. Cognitive Funct. & Pathol., Grad. Sch. Med. Sci., Nagoya City Univ.*

Selecting an appropriate behavior is critical for survival in conflict situations during which animals face both appetitive and aversive stimuli. Animals in conflict situations exhibit several processes. First, they remain still in a safe place (suspension); once they decide to take action, they assess risks at a place where danger may occur (risk assessment) and finally reach the reward. However, most studies have not addressed these conflict processes. We developed a new experimental paradigm – the three-compartment conflict task – to quantitatively evaluate conflict processes. Our apparatus consisted of start, flat, and grid compartments. Mice were required to explore the grid compartment where they might receive foot shocks while trying to obtain sucrose. We found that applying foot shocks increased sucrose acquisition latency in the subsequent trials, reflecting an elevated conflict level throughout the trials. Time spent in the start compartment and the number of retreats were measured as parameters for conflict levels in suspension and risk assessment, respectively. Both parameters were increased by foot shocks. Treatment with diazepam decreased these parameters. Our new paradigm is a valuable tool for quantitatively evaluating conflict processes and contributes to the development of effective treatments for psychiatric disorders associated with maladaptive behaviors in conflict situations.

1-B-YIA3-2 一般演題（YIA）

# Characteristics of depression model mice produced by repeated administration of dexamethasone after lipopolysaccharide-induced inflammation

Fumiya Shibagaki, Naoko Kojima, Akane Furukawa, Noritaka Nakamichi

*Lab. Mol. Pharmacother., Fac. Pharm., Takasaki Univ. Health and Welfare*

Although animal models of depression are produced by loading chronic stress, inducing neuroinflammation, or administering drugs that induce depression, the results obtained in these models have poor reproducibility. Therefore, it is necessary to develop animal models that exhibit definitive symptoms of depression for studies on potential therapeutics. This study aimed to investigate depressive symptoms and their pathogenesis in lipopolysaccharide (LPS)-inflamed mice treated with dexamethasone (DEX). Male ICR mice were injected with LPS, followed by injection with DEX at day later once daily for 6 days. Mice in the LPS+DEX group had significantly longer immobility time in the tail-suspension and forced swim tests than did those in the LPS or DEX only and control groups at 7 days post-LPS administration. In immunohistochemical analysis, significantly lower number of the immature neuronal marker doublecortin-positive cells were observed in the hippocampal dentate gyrus of mice in the LPS+DEX group compared with those of mice in the LPS or DEX only and control groups at 7 days after LPS administration. These results suggest that repeated DEX administration to LPS-inflamed mice may induce definitive symptoms of depression by decreasing the number of immature neurons in the hippocampal dentate gyrus.

1-B-YIA3-3 一般演題（YIA）

# The effect of diazepam on aggressive biting behavior of isolation-reared ddY mouse is different between male and female.

Kento Igarashi[1,2], Satoshi Kuchiiwa[3], Toshiko Kuchiiwa[4], Koh-ichi Tanaka[1,2], Junichi Kitanaka[2], Nobue Kitanaka[5], Nobuyoshi Nishiyama[2], Kazuo Tomita[1,2], Tomoaki Sato[1]

*[1]Dept. Applied Pharmacol., Grad. Sch. Med. & Dent. Sci., Kagoshima Univ., [2]Dept. Pharm., Sch. Pharm., Hyogo Med. Univ., [3]Dept. Morphol. Sci., Field of Neurol., Grad. Sch. Med. & Dent. Sci., Kagoshima Univ., [4]Dept. of Clinical Pshychol., Grad. Sch. Human Sci., Kagoshima Immaculate Heart Univ., [5]Dept. Pharmacol., Sch. Med., Hyogo Med. Univ.*

[Background and Purpose] A mouse aggressive response meter (ARM) is a device that measures aggressive biting behavior (ABB) toward a metal rod presented in front of a mouse. With this device, we have previously found that kamishoyosan, a Japanese traditional herbal medicine, reduces this aggressive behavior (Igarashi et al., Brain Res. 2021). Although, in the previous study, it was suggested that males and females have different effects on GABAA receptors, the details have not been clarified. In this study, we investigated the effects of diazepam, an agonist for GABAA receptors.

[Experimental methods and results] Male and female ddY mice were isolation-reared from 3 to 10 to 11 weeks after birth. Isolation-reared male mice showed a 51% decrease in ABB 1 hour after i.p. administration with 2 mg/kg diazepam ($p<0.05$). On the other hand, no significant changes were observed in female mice treated with 2mg/kg diazepam. ABB was reduced by approximately 63% in male and 53% in female mice treated with 4 mg/kg diazepam ($p<0.05$). Next, we examined the expression level of potassium-chloride ion transporter (KCC2) mRNA in the prefrontal cortex by real-time PCR, and we found that it was decreased to about 8% in isolation-reared female mice compared to group-reared female mice ($p<0.05$). No significant difference was found between isolated and group-housed male mice.

[Discussion] KCC2 contributes inhibitory neural transmission by exporting intracellular chloride ion (Cl-). In this study, KCC2 expression in the PFC was decreased only in isolated female mice, which may disturb inhibitory neural transmission, consequently make it difficult for diazepam to reduce aggression.

1-B-YIA3-4 一般演題（YIA）

# Involvement of fatty acid-binding protein 3 in the mechanism for exacerbation of postoperative pain of high fat-induced obesity model mice

Dan Tachibana, Kazuo Nakamoto, Shogo Tokuyama

*Dept. Clin. Pharmacy, Sch Pharmaceu. Sci., Kobe Gakuin Univ.*

Obesity is said to be one of the exacerbating factors of chronic pain, however its mechanism is unclear. Recently, fatty acid binding protein 3 (FABP3) functions as not only an intracellular chaperone to transport fatty acids, but also the signal transduction and gene transcription. There are some recent reports that FABP3 is induced in response to an increased dietary fat load as well as obesity, inflammation and pain. Here we tested whether FABP3 involve in the mechanism for obesity-induced exacerbation of postoperative pain using FABP3 deficit (FABP3KO) mice. Male ddY and C57BL6J wild-type (WT) mice were used by experiment. WT and FABP3 KO were fed on control diet or high fat diet (HFD) for 8 weeks. Postoperative pain was induced by paw incision. Mechanical allodynia was evaluated by von Frey test. Mice with paw incision showed mechanical allodynia. Repeated intracerebroventricully injection of FABP-IN-1, a FABP inhibitor for FABP3, 5 and 7, suppressed paw incision-induced mechanical allodynia. The mice fed HF diet exacerbated paw incision-induced mechanical allodynia compared to those in control diet fed WT mice. On the other hand, FABP3KO mice fed HF diet suppressed paw incision-induced mechanical allodynia. Our findings suggest that FABP3 might at least in part involve in obesity-induced exacerbation of postoperative pain.

**1-B-YIA3-5** 一般演題（YIA）

# Detection of the proteins in a unique extracellular fluid of the mouse inner ear

Fukuda Masatoshi[1,2], Hiroki Okanishi[3], Daisuke Ino[1], Eri Wakai[1], Yumi Ohta[2], Takashi Sato[2], Hidenori Inohara[2], Yoshikatsu Kanai[3], Hiroshi Hibino[1]

*[1]Division of Glocal Pharmacology, Department of Pharmacology, Graduate School of Medicine, [2]Department of Otorhinolaryngology, Graduate School of Medicine, Osaka University, [3]Department of Bio-syastem Pharmacology, Graduate School of Medicine*

In Japan, 10% of the population suffer from hearing loss, which impairs daily life; it is an urgent issue to identify the causative factors and develop the innovative therapies. Irreversible hearing loss stems primarily from disorders of the cochlea of the inner ear. The cochlea consists of three tubular structures. Two tubules contain perilymph, which exhibits an ionic composition similar to plasma. Another tubule is filled with endolymph, which shows a highly positive potential of +100 mV. This unique environment sensitizes hearing. Because the endolymph is packed in the narrow space and its quantity is small of ~1 μL in a mouse cochlea, the protein content remains unclear. To address this challenge, we developed a sophisticated approach to collect the endolymph from the cochlea of live mice. We fabricated a micropipette filled with a conductive organic solvent and inserted it into the cochlea, while monitoring the potential. As detecting the high potential of the endolymph, we aspirated the fluid into the pipette. The sample was then analyzed by LC-MS/MS. When comparing the result to the profile of the perilymph, we found that a few proteins including molecules involving lipid metabolism were enriched in the endolymph. The protein data described here may be useful for understanding of the mechanisms underlying hearing loss.

**2-B-YIA4-1** 一般演題（YIA）

# Finerenone-induced cardioprotective effects associated with the suppression of myocardial sodium buildup in salt/aldosterone-loaded uninephrectomized rats

Rahman Asadur[1], Tatsuya Sawano[2], Kento Kitada[1], Takeshi Imamura[2], Akira Nishiyama[1]

*[1]Department of Pharmacology, Faculty of Medicine, Kagawa University, Japan, [2]Division of Pharmacology, Faculty of Medicine, Tottori University, Japan.*

Finerenone, a nonsteroidal mineralocorticoid-receptor blocker, has been shown in clinical trials to have considerable cardioprotective benefits. However, its precise mechanism is not clear. Here, we aimed to test the hypothesis that cardioprotective effects of finerenone are linked to a decrease in salt buildup and, as a result, a lower macrophage chemotaxis and/or a change in its phenotype in heart tissues. First, effects of finerenone (10 mg/kg body weight by oral gavage) on myocardial injury and sodium accumulation were examined in uninephrectomized (UNx) Sprague-Dawley rats with chronic aldosterone infusion (0.75 μg/hr) and salt-loading through drinking water (1% NaCl) for 4 weeks. Echocardiography and gene expression analyses revealed an adverse cardiac remodeling as well as diastolic dysfunction with preserved ejection fraction. Notably, finerenone treatment completely prevented the cardiac dysfunction with the improved cardiac remodeling in these rats. Furthermore, sodium content in left ventricular tissues were markedly elevated in salt-loaded aldosterone-infused UNx rats, but significantly reduced in rats with concomitant finerenone treatment. Moreover, gene expression of F4/80 (a macrophage marker) was significantly reduced by finerenone treatment. Apart from that, finerenone dramatically reduced the salt-induced elevation in M1 markers (TNF-alpha and iNOS) in RAW264.7 cells, whereas M2 markers remained unaltered. These data indicate that finerenone has the potential to mitigate cardiac dysfunction in salt-loaded and aldosterone-infused rats by suppressing sodium accumulation in left ventricular tissues. These effects of finerenone may attenuate the subsequent inflammation by macrophages and adverse cardiovascular remodeling.

withdrawal

**2-B-YIA4-3** 一般演題（YIA）

# Vascular endothelial dysfunction is involved in the fluoroquinolone-associated aortic aneurysm and dissection

Miyata Koji[1], Yuki Izawa-Ishizawa[1,2], Honoka Nishi[1], Shuto Itokazu[1], Tatsumi Miyata[1], Kaito Tsujinaka[1,3], Masateru Kondo[1,3], Takahiro Niimura[1,4], Fuka Aizawa[1,3], Kenata Yagi[1,4], Kei Kawada[1,5], Mitsuhiro Goda[1,3], Keisuke Ishizawa[1,3,4]

*[1]Dept. Clin. Pharmacol. & Therap., Tokushima Univ., [2]Dept. Gen. Med., Taoka Hosp., [3]Pharm., Tokushima Univ. Hosp., [4]Clin. Res. Ctr. Dev. Therap., Tokushima Univ. Hosp., [5]Dept. Pharm. Pract. Pedagog., Tokushima Univ.*

Aortic aneurysm and dissection (AAD) are common arterial diseases with high mortality. Recently, AAD have been reported as adverse events associated with fluoroquinolones. Previous studies showed fluoroquinolones could induce AAD through arterial media disorder, but the influence of fluoroquinolones to vascular endothelium is unknown. The aim of current study is to evaluate the effect of fluoroquinolone to vascular endothelium on the development of AAD. We analyzed the association in clinical situation between fluoroquinolones and AAD using VigiBase, the World Health Organization's global database. We evaluated whether levofloxacin (LVFX) affects the AAD development using human umbilical vein endothelial cells (HUVECs) and aortic dissection model mice which induced by three drugs; nitric oxide synthase inhibitor, angiotensin II, and lysyl oxidase inhibitor. VigiBase analysis showed that the fluoroquinolones had the association of high reporting rate of AAD. Although LVFX did not significantly increase the incidence of AAD in mice, LVFX treatment increased VCAM-1 expression in HUVECs. Our results suggested that the endothelial dysfunction might be one candidate mechanism of fluoroquinolone-associated AAD.

**2-B-YIA4-4** 一般演題（YIA）

# RAMP1 signaling attenuates acute lung injury by inhibiting cytokine production and neutrophil recruitment.

Yamashita Atsushi[1,2], Yoshiya Ito[1], Hiromi Matsuda[2], Mayuko Osada[1], Seishiro Akinaga[1], Mariko Kamata[1], Kanako Hosono[1], Ko Hatanaka[1], Masataka Majima[1,3], Hirotsugu Okamoto[2], Hideki Amano[1]

*[1]Dept. Pharm., Sch. Med., Kitasato Univ., [2]Dept. Anesthesiology, Sch. Med., Kitasato Univ., [3]Fac. Health & Med. Sci., Kanagawa Inst. Tech.*

Purpose: Calcitonin gene-related peptide (CGRP) regulates inflammation through receptor activity-modifying protein 1 (RAMP1), a subunit of CGRP receptor complex in immune cells. Acute respiratory distress syndrome (ARDS) is a severe respiratory dysfunction induced by cytokine storm that leads to alveolar epithelial damage and increased pulmonary vascular permeability. This study investigated the role of RAMP1 signaling in the pathology of ARDS.

Methods and Results: ARDS was created by an intratracheal administration of lipopolysaccharide (LPS) to male RAMP1-knockout (RAMP1-/-) mice and wild-type (WT) mice. As compared with WT, RAMP1-/- exhibited increases in fatality rates, infiltration of inflammatory cells and hemorrhage in the lung tissues, and levels of total protein, inflammatory cytokines (TNF$\alpha$, IL-1$\beta$ and, IL-6), and chemokine (CXCL12) in bronchoalveolar lavage fluid (BALF). RAMP1 was expressed in alveolar macrophages (AMs), and CGRP levels in BALF were increased in WT and RAMP1-/- at 72 h. Removal of AMs with clodronate liposome (CL) enhanced lung injury and total protein levels, and reduced cytokines (TNF$\alpha$ and IL-1$\beta$) in both genotypes at 6 h, but increased the cytokines and CXCL2 together with neutrophil accumulation at 72 h. Cultured AMs from RAMP1-/- showed higher levels of cytokines and chemokines than those in WT.

Conclusion: These results suggested that deletion of RAMP1 signaling in AMs aggravated LPS-induced acute lung injury by increasing vascular permeability, inflammatory cytokines production, and neutrophil accumulation.

**2-B-YIA4-5** 一般演題（YIA）

# Overexpression of aquaporin-5 (AQP5) in pulmonary epithelial cells suppresses sepsis-induced edema by inhibiting epithelial apoptosis

Ishii Shinya, Yuta Uchiyama, Kazuhito Murakami, Yoichiro Isohama

*Lab. of appl. pharmacol., Fac. of Pharm. of Sci., Tokyo Univ. of Sci.*

The development of acute respiratory syndrome (ARDS) during sepsis clinically doubles the mortality rate of patients. ARDS is essentially non-cardiogenic pulmonary edema, based on increasing permeability of vascular endothelial and pulmonary epithelial cells due to their injury and cell death caused by excessive inflammation in lung. It has been also known that the expression of AQP5 in lung tissues is considerably reduced in LPS induced ARDS mouse model. Therefore, it is possible that the decrease in AQP5 expression contributes to the pathogenesis of ARDS. We have established a transgenic (Tg) mouse in which AQP5 is highly expressed specifically in the pulmonary epithelial cells. In this study, we have examined the significance of changes in AQP5 expression in ARDS, using this Tg mice. In wild type (WT) mice, intraperitoneal treatment of LPS decreased survival rate and caused pulmonary edema evaluated by lung wet/dry weight ratio. In AQP5-Tg mice, the decrease in survival rate and pulmonary edema caused by LPS was considerably improved. In addition, TUNEL stained lung tissue revealed that the apoptotic cells in AQP5-Tg mice was significantly less than that in WT mice. These results indicated that the decrease in AQP5 expression in ARDS enhances the apoptosis of pulmonary epithelial cells and exacerbate pulmonary edema.

2-B-YIA5-1 一般演題（YIA）

# Pemigatinib suppresses liver fibrosis and subsequent osteoporosis

Mihara Taiki[1], Yoshiharu Tsuru[2], Tamaki Kurosawa[1], Yuma Nonoshita[1], Yuki Yamakawa[1], Masatoshi Hori[1]

*[1]Laboratory of Veterinary Pharmacology, Graduate School of Agriculture and Life Sciences, The University of Tokyo, [2]Research Support Dev., PRIMETECH Corp.*

Background & Aims

Liver fibrosis could lead to fatal secondary diseases such as cirrhosis and hepatocellular carcinoma, including osteoporosis. However, there are no effective treatments for liver fibrosis and subsequent osteoporosis, necessitating new therapeutic targets. Recently, fibroblast growth factor 23 (FGF23) has garnered attention as a potential fibrosis-promoting factor. FGF23 also controls the phosphorus level in the body; excess FGF23 level causes phosphorus deficiency, resulting in impaired bone microstructure. In this study, we hypothesized that the FGF23 level increases with liver injury, which in turn induces liver fibrosis and osteoporosis.

Results

We found that carbon tetrachloride-induced liver injury increased the serum FGF23 level. RNA sequencing analysis using FGF23-treated hepatic stellate cells showed that FGF23 promotes the production of Matrisomes, which helps form the extracellular matrix. The FGF receptor antagonist pemigatinib alleviated carbon tetrachloride-induced liver fibrosis and dysfunction. Moreover, pemigatinib suppressed the deleterious alterations in bone density and microstructure.

Conclusion

We found that the serum FGF23 level increased with liver injury, FGF23 promoted liver fibrosis, and inhibition of FGF23–FGFR signaling alleviated liver fibrosis and subsequent osteoporosis. These findings suggest that FGF23–FGFR signaling may be a new therapeutic target for liver fibrosis and subsequent osteoporosis.

# Thromboxane $A_2$ receptor signaling in macrophages promotes liver repair after acetaminophen-induced liver injury

Mina Tanabe[1], Yoshiya Ito[1], Mayuko Osada[1], Takuya Yamazaki[1], Yu Kuroda[1], Mariko Kamata[1], Kanako Hosono[1], Kou Hatanaka[1], Masataka Majima[2], Hideki Amano[1]

*[1]Dept. Pharm., Sch. Med., Kitasato Univ., [2]Fac. Health &Med. Sci., Kanagawa Inst. Tech.*

**Objective:** Acetaminophen (APAP) overdose causes severe acute liver failure. Impaired liver repair and regeneration after APAP hepatotoxicity leads to failed recovery and mortality. Accumulating evidence indicates that macrophages play a critical role in liver repair after APAP-induced liver injury; however, underlying mechanisms of involvement of macrophages remain unknown. Here, we examined the role of endogenous thromboxane $A_2$ ($TXA_2$) in macrophages in liver repair after APAP-induced liver injury.

**Methods and Results:** APAP (300 mg/kg, ip) was administered to macrophage-specific thromboxane prostanoid receptor (TP) deficient mice (mTPKO) and control mice (Cont). Compared with Cont, mTPKO exhibited severe liver injury as indicated by increased levels of ALT, necrotic area, and pro-inflammatory cytokines (TNF-$\alpha$, IL-1$\beta$, and IL-6) and decreased expression of PCNA, a marker of hepatocyte proliferation at 48 h after APAP treatment. CD68-positive macrophages less accumulated in the livers from mTPKO, accompanied by reduced expressions of chemokines. Flow cytometry analysis revealed that the numbers of M1 macrophages in mTPKO were higher than control, while the numbers of M2 macrophages in mTPKO were lower than control. In cultured bone marrow-derived macrophages from mTPKO, M1-related gene expressions were increased and M2-related gene expressions were decreased.

**Conclusions:** TP receptor signaling in macrophages promoted liver repair after APAP-induced liver injury by accumulating M2 macrophages in the livers.

**2-B-YIA5-3** 一般演題（YIA）

# Sphingosine Kinase 1 Aggravates Liver Fibrosis by Mediating Macrophage Recruitment and Polarization

Tian Lan[1], Xiang Zhang[2], Shiyun Chen[1], Xin Ding[1]

[1]*Guangdong Pharmaceutical University,* [2]*The Chinese University of Hong Kong*

Macrophage recruitment and polarization play pivotal roles in the initiation and progression of liver fibrosis. Our previous study has demonstrated that Sphingosine kinase 1 (SphK1) has distinct roles in the activation of Kupffer cells (KCs) and hepatic stellate cells (HSCs) in liver fibrosis. However, the role of SphK1 in hepatic macrophage recruitment and polarization remains unclear. In this study, Single-cell transcriptomics illustrated that SphK1 is highly expressed in monocytes/macrophages and upregulated during both stages of macrophage M1 and M2 polarization. Consistently, SphK1 expression was elevated and positively correlated with macrophage M1 and M2 polarization in human fibrotic livers. SphK1 deletion reduced the recruitment of hepatic macrophages and inhibited M1 and M2 polarization in $CCl_4$-induced mice. SphK1 deficiency in endogenous liver cells attenuated macrophage recruitment via CCL2. SphK1 in macrophage activated the ASK1-JNK1/2-p38 signaling pathway to promote M1 polarization. Furthermore, macrophage SphK1 downregulated small ubiquitin-like modifier (SUMO) specific peptidase1 (SENP1) so as to decrease de-SUMOylation of Kruppel-like factor 4 (KLF4) to promote M2 polarization. Together, our findings highlighting that SphK1 aggravated liver fibrosis by promoting macrophage recruitment and polarization and might serve as a potential drug target for the treatment of liver fibrosis.

2-B-YIA5-4 一般演題（YIA）

# Establishment of gall bladder organoid derived from cholesterol-induced cholelithiasis model mouse

Yamamoto Haru, Maria Mochizuki, Tatsuya Usui, Kazuaki Sasaki

*Lab. Vet Pharm., Dept Vet Med., Tokyo Agri. & Tech. Univ.*

【Background】Cholesterol-induced cholelithiasis is the most popular diseases in all of cholelithiasis. Cholesterol gall stones are generally formed caused by various factor following unbalance cholesterol secretion. Recently, it has been reported that there are some functional changes of gall bladder like mucus secretion or motility as one of the factors for cholelithiasis, but there has not been established the mechanism related to the functional changes of gall bladder mucosa. Additionally, organoid is often used for the pathological evaluation because it can reproduce cell composition, characteristics, and function, but it has not been established organoid model for cholelithiasis.

【Objectives】We established gall bladder organoid derived from cholesterol-induced cholelithiasis model mouse and evaluated the functional disorder mechanism for cholesterol-induced cholelithiasis.

【Methods】We feeded inducing diet to 4 weeks or 8 weeks mice, and produced cholesterol-induces cholelithiasis model mice. Using extracted tissue, we evaluated the pathological characteristics about gall stones formation and gall bladder structure. And also, we cultured gall bladder organoid derived from extracted tissue and compared its structure or mucus production with original tissue. Additionally, the gene expression difference was analyzed by RNA sequence.

【Results】It was observed the gall stones formation in 4 weeks feeding mouse. Organoid size in 4 weeks feeding mouse was significantly bigger than control group. In 8 weeks feeding mouse, organoid size was also increased significantly and gallbladder inflammation or gallbladder wall hypertrophy were recognized in gallbladder tissue. Furthermore, in RNA sequence, there were genetic expression differences between control and feeding group.

【Conclusion】It was suggested that gall bladder organoid derived from cholesterol-induced cholelithiasis model mouse can reproduce pathological characteristics of cholelithiasis. We will plan to do more functional analysis based on RNA sequence.

# Nivolumab receptor occupancy on effector regulatory T cells predicts clinical benefit

Hosonuma Masahiro[1,2,3,4], Yuya Hirasawa[4], Atsuo Kuramasu[2], Yuta Baba[2], Toshiaki Tsurui[1,2,3], Takuya Tsunoda[4], Yuji Kiuchi[1,2,3], Kiyoshi Yoshimura[2,4]

*[1]Division of Medical Pharmacology, Department of Pharmacology, Showa University School of Medicine, [2]Department of Clinical Immuno Oncology, Clinical Research Institute for Clinical Pharmacology and Therapeutics, Showa University, [3]Pharmacological Research Center, Showa University, [4]Division of Medical Oncology, Department of Medicine, Showa University School of Medicine*

Immune checkpoint inhibitor discovery represents a turning point in cancer treatment. However, the response rates of solid tumors remain approximately 10%–30%; consequently, prognostic and immune-related adverse event (irAE) predictors are being explored. The programmed cell death protein 1 (PD-1) receptor occupancy (RO) of PD-1 inhibitors depends on the number of peripheral blood lymphocytes and their PD-1 expression levels, suggesting that the RO may be related to efficacy and adverse events. As PD-1 inhibition affects each T-cell subset differently, the RO of each cell population must be characterized. However, relevant data have not been reported, and the prognostic relevance of this parameter is not known. In this study, we aimed to clarify the association between the nivolumab RO in each T-cell population and patient prognosis and reveal the development of irAEs in nivolumab-treated patients. Thirty-two patients were included in the study, and the mean follow-up period was 364 days. The nivolumab RO on effector regulatory T cells (eTregs) was significantly lower in the group that presented clinical benefits, and a significant negative association was observed between PD-1 occupancy on eTregs and all-cause mortality. The results suggest that the nivolumab RO on eTregs may be a prognostic factor in PD-1 inhibitor therapy, implying that the inhibition of PD-1/PD-ligand 1 (PD-L1) signaling on eTregs may attenuate antitumor effects.

# CCR5$^{+}$ cells possibly contribute to development of lung fibrosis in asthma

Shimora Hayato, Yukino Nagatani, Itomi Takamori, Keitaro Nishikawa, Masaya Matsuda, Kazuyuki Kitatani, Takeshi Nabe

*Lab. of Immunopharmacol., Fac. of Pharm. Sci., Setsunan Univ.*

Lung fibrosis is developed in severe asthmatic patients, whereas the mechanisms are unclear. We have established a murine model of steroid-insensitive asthma, which shows lung fibrosis. RNA-seq analyses revealed genes encoding CCR5 and its ligands were upregulated in the lung. However, roles of CCR5 in the development of fibrosis were unclear. In this study, we analyzed whether a CCR5 antagonist, maraviroc improves the fibrosis and increase in one of profibrotic cell types, monocyte-derived alveolar macrophages (MoAMs). OVA-sensitized BALB/c mice were intratracheally challenged with OVA. Dexamethasone (DEX, 1 mg/kg i.p.) or maraviroc (50 mg/kg p.o.) was administered during the challenges. The development of lung fibrosis and the number of MoAMs (CD45$^{+}$ CD64$^{+}$ Ly-6C$^{+}$ Ly-6G$^{-/low}$ Siglec-F$^{-}$) in the lung were analyzed. Maraviroc improved the development of lung fibrosis, whereas DEX did not affect it. Increase in MoAMs was suppressed by neither DEX nor maraviroc. Yet, interestingly, the number of CCR5$^{+}$ cells were decreased in the maraviroc-treated mice. The decreased CCR5$^{+}$ cells expressed CD45, CD11b, Ly-6C and Ly-6G, which displayed phenotype of monocyte-derived suppressor cells (MDSC). It was suggested that CCR5 possibly contributes to the increase in CCR5$^{+}$ MDSC-like profibrotic cells, leading to the development of lung fibrosis.

**2-B-YIA6-2** 一般演題（YIA）

# AMPK/mTOR signaling pathway attenuates subtype-selective differentiation of Myeloid-Derived Suppressor Cells (MDSC)

Sugiyama Shintaro, Kazuhito Murakami, Yoichiro Isohama

*Lab. of appl. Pharmacol., Fac. of Pharm. of Sci., Tokyo Univ. of Sci.*

Myeloid-Derived Suppressor Cells (MDSCs) are generated during tumor-bearing condition, and inhibit T-cell activity to promote cancer growth. Therefore, drugs which can inhibit MDSCs are new predictive immunotherapeutic medicines. On the other hand, AMP-activated protein kinase (AMPK) and mTOR signaling plays not only important role in energy homeostasis, but also recently revealed to plays a central role in an anti-tumor response. In this study, we examined the effect of metformin, an AMPK activator, and rapamycin, an inhibitor of mTOR on MDSCs differentiation. Bone marrow cells were isolated from of C57BL/6J mice and differentiated into MDSCs by the treatment with IL-6 and GM-CSF. Both metformin and rapamycin dose-dependently decreased differentiated MDSCs. These drugs also decreased the suppressing ability of MDSCs on T cell proliferation. Interestingly, subtype analysis of MDSC has shown that, these drugs decreased the ratio of monocytic MDSCs (M-MDSCs), whereas that of granulocytic MDSCs (G-MDSCs) was increased. Taken together, we assume that activation of AMPK and following inhibition of mTOR selectively inhibits M-MDSCs differentiation, and their immunosuppressive property. We expect that this study will provide new insights into the pharmacological regulation tumor immunology.

2-B-YIA6-3 一般演題（YIA）

# Ferroptosis induced by eribulin and its mechanism in ovarian cancer cells

Mana Azumi[1], Mikihiro Yoshie[1], Kazuya Kusama[1], Saya Nakano[1], Atsuya Tsuru[1], Tomoyasu Kato[1,2], Kazuhiro Tamura[1]

*[1]Dept. Endocrine Pharmacol. Tokyo Univ. Pharmacy and Life Sci.,[2]Div. Gynecol., Natl. Cancer Ctr. Hosp.*

Ovarian cancer is a gynecologic malignancy with a high mortality rate. Eribulin, a non-taxane microtubule inhibitor approved for breast cancer and sarcoma, exerts antitumor efficacy in ovarian cancer cells (author et al, BPB. 2022:45). Ferroptosis, an iron-dependent cell death resulting from lipid peroxidation, is triggered by an accumulation of intracellular iron leading to oxidative stress. Reactive oxygen species (ROS) are a cause of oxidative stress, and crucial for mitochondrial homeostasis. We explored the involvement of ferroptosis and its mechanism in the antitumor activity of eribulin in ovarian cancer cells (RMG-1). Eribulin-induced cell death was mitigated by deferoxamine, an iron chelator. Eribulin elevated the levels of intracellular iron, lipid peroxides, ROS, and mitochondrial membrane potential. Eribulin downregulated NRF2, heme oxygenase-1 (HO-1) and dihydroorotate dehydrogenase (DHODH), whereas glutathione peroxidase (GPX4) protein level remained unaffected. Combining eribulin with ML210, a GPX4-inhibiting ferroptosis inducer, enhanced eribulin-induced cell death. Taken together, eribulin triggers ferroptosis characterized by increased intracellular iron, lipid peroxidation, and ROS in ovarian cancer cells. The ferroptosis-inducing effect may be orchestrated through suppression of the NRF2/HO-1 signaling pathway and lipid peroxidation inhibition by DHODH. These findings illuminate the potential of eribulin-induced ferroptosis as a therapeutic strategy in ovarian cancer treatment.

# Mechanism and pathological significance of cysteine metabolic reprogramming associated with hepatocarcinogenesis

Yamauchi Tomoaki[1], Yumi Okano[1], Daisyu Terada[1], Akito Tsuruta[1,2], Satoru Koyanagi[2], Shigehiro Ohdo[1]

*[1]Dept. of Pharmaceutics, Grad. Sch. Pharm., Kyushu Univ., [2]Dept. Glocal Healthcare Sci., Grad. Sch. Pharm., Kyushu Univ.*

Many types of cancer cells have increased demand for specific amino acids, depending on either exogenous supply or upregulated de novo synthesis. Intracellular accumulation of cysteine (Cys) is often observed in cancer cells, which is thought to contribute to the elimination of oxidative stress associated with rapid cell proliferation and/or exposure to anticancer drugs. However, the mechanism of metabolic reprogramming of cysteine during the oncogenesis is not fully understood yet. In this study, we found that the expression levels of genes responsible for cysteine synthesis were downregulated in the hepatocarcinoma-formed tumor tissues implanted in mice. On the other hand, the expression levels of cystine uptake transporter, xCT, were increased in hepatic tumor tissues as compared with healthy liver. The expression of DNA methyltransferase was also increased in hepatocarcinoma tumor tissues and caused DNA methylation of cysteine synthesis genes thereby repressing their expression. Pharmacological inhibition of cysteine synthesis resulted in a temporal decrease in intracellular cysteine contents and upregulation of xCT expression. Therefore, reduction of intracellular cysteine levels appeared to repulsively increase Cys uptake via promoting xCT expression. These findings suggest that the accumulation of Cys in hepatocarcinoma tumor cells results from enhancement of exogenous supply. This metabolic reprogramming may be required for the survival ability of oncogenic transformed cells.

**2-B-YIA6-5** 一般演題（YIA）

# Development of deep learning methods for multiple mice tracking

Naoaki Sakamoto[1], Hitoshi Kakeno[1], Noriko Ozaki[1], Yusuke Miyazaki[1], Koji Kobayashi[2], Takahisa Murata[1,2,3]

*[1]Dept. Animal Radiolody., Grad. Agr. & Life Sciences. Univ of Tokyo,[2]Crs. Food and Animal Systemics., Grad. Agr. & Life Sciences. Univ of Tokyo,[3]Dept. Veterinary Pharmacol, Grad. Agr. & Life Sciences. Univ of Tokyo*

Experimental animals including mice interacts with others and exhibits variety of behaviors. However, conventional behavioral tests mostly focused on single mouse behavior since visual tracking for multiple mice is practically impossible. Here, we aimed to develop the tracking tool for multiple mice using deep learning methods for image recognition. Behaviors of two to four C57BL/6 mice were recorded with handy camera in an open field arena. First, we manually labeled the mouse contours for hundreds of frame images and trained a deep learning model with labeled images. Next, mouse counters in all frames were predicted by the trained model, and assigned IDs by calculating similarities of every pair of contours between frames. Finally, we tracked the geometric center of contours that has the same IDs and semi-automatically corrected predictive errors to improve the performance. The established system could accurately track two to four mice under light conditions. In addition, we confirmed that this system accurately predicted the videos with bedding in the arena and could evaluate the videos recorded with infrared lights. This technology provides a new approach to evaluate mouse behaviors in pharmacological research.

1-B-O01-1 一般演題（口頭）

# Crystal structures of monoamine oxidase-B with PET ligands [$^{18}$F]SMBT-1/ [$^{18}$F]THK5351

Ryuichi Harada[1,2], Kaede Goto[3], Kaede Kudo[2], Yukitsuka Kudo[2], Takeshi Yokoyama[3], Shozo Furumoto[4], Nobuyuki Okamura[5], Yoshikazu Tanaka[3]

*[1]Tohoku Univ. Sch.Med. Pharmacol.,[2]Tohoku Univ. IDAC,[3]Tohoku Univ. Sch. Life.,[4]Tohoku Univ. CYRIC,[5]Tohoku Med. and Pharm. Univ. Sch. Med. Pharmacol.*

Monoamine oxidase-B (MAOB) is a crucial enzyme not only as a therapeutic target for Parkinson's disease but also as a binding target for PET tracers that image reactive astrogliosis. We have developed a PET tracer named [$^{18}$F]SMBT -1 designed for in vivo MAOB imaging. This was achieved by altering the chemical structure of tau PET tracer THK5351, which had high affinity for MAOB. To better understand the interaction of MAOB with these PET tracers, we determined the atomic structure of MAOB-SMBT-1 and MAOB-THK5351 complexes by X-ray crystallography with soaking method. Initially, we confirmed the high binding affinity of [$^{18}$F]SMBT-1 against purified MAOB ($K_D$ = 4.4, $B_{max}$ = 2.8 nmol/mg protein), which was consistent with previously determined values against commercially available microsomal MAOB ($K_D$ = 3.7 nM, $B_{max}$ = 0.11 nmol/mg protein). *In vitro* competitive binding and biochemical assays demonstrated that SMBT-1 and THK5351 possess higher binding affinity and enzymatic inhibitory activity against MAOB than clinically used MAOB inhibitors Rasagiline and Safinamide. The crystal structures of MAOB in complexes with SMBT-1 and THK5351 (determined at 2.25 - 2.6Å) showed that they bind in the active site cavity of the protein in front of the flavin adenine dinucleotide cofactor. The findings achieved in this study would provide a potentially practical guide to avoid off-target binding to MAOB or to develop higher potency radiopharmaceuticals.

1-B-O01-2 一般演題（口頭）

# A trans-omic analysis of metformin action in the liver

Katsuyuki Yugi

*RIKEN IMS*

Metformin is a drug for type 2 diabetes used as the first-line mainly in North America and Europe. Its mechanism of action is believed to lower blood glucose levels by inhibiting gluconeogenesis in the liver and increasing glucose consumption in peripheral tissues. However, there are still controversies regarding the target molecules and the biochemical networks that induce pharmacological actions downstream of the target molecules. We applied the methodology of trans-omics analysis, which elucidates metabolic regulatory mechanisms as a large-scale molecular network, to characterizing the mechanism of action of metformin in the liver. We will show comprehensive identification of target proteins and reconstruction of metabolic regulatory networks that induce pharmacological actions of metformin.

1-B-O01-3 一般演題（口頭）

# Sialic acid degradation in the inflamed skin attenuates the number of the epidermal nerve fibers and inflammatory pain via calcitonin gene related peptide receptors

Shun Watanabe[1,2], Ruka Saito[1,2], Marie Abe[1,2], Misa Oyama[1,2], Takashi Iwai[1,2], Mitsuo Tanabe[1,2]

*[1]Lab. Pharmacol., Sch. Pharmaceut. Sci., Kitasato Univ., [2]Med. Res. Lab., Sch. Pharm., Kitasato Univ.*

The number of nerve fibers in the epidermis is increased during skin inflammation, which causes inflammatory pain. Sialyl glycosphingolipid, ganglioside, regulates axonal elongation and maintains axon morphology. Our previous study showed that *Arthrobacter ureafaciens* sialidase that degrades sialyl conjugates attenuated the number of epidermal nerve fibers and inflammatory pain. Moreover, F-11 cells derived from the dorsal root ganglion neuron treated with sialidase showed reduced neurite length and enhanced calcitonin gene-related peptide (CGRP)-immunoreactivity.
Thus, we investigated the effects of olcegepant, a CGRP receptor antagonist, on the inhibition of epidermal nerve fibers and inflammatory pain when treated with sialidase. One day after intraplantar injection of complete Freund's adjuvant into the mouse hind paw to initiate skin inflammation, olcegepant and sialidase were injected into inflamed skin. The number of nerve fibers in the epidermis was counted using immunofluorescent staining with anti-PGP 9.5 antibody. Olcegepant attenuated the analgesic effects and epidermal nerve fiber collapse by sialidase. These results suggested that sialidase degenerated epidermal nerve fibers in inflamed skin via CGRP receptor activation, resulting in analgesia.

1-B-O01-4 一般演題（口頭）

# Increasing CD11c+ microglia cells facilitates the resolution of neuropathic pain behavior

Kohno Keita, Ryoji Shirasaka, Keita Hirose, Yuto Shibata, Makoto Tsuda

*Department of Molecular and System Pharmacology, Graduate School of Pharmaceutical Sciences, Kyushu University*

Neuropathic pain is a pathological pain state caused by a lesion or disease affecting the somatosensory system. Because existing analgesics often do not work, the development of new drugs for neuropathic pain is needed. A mouse model of neuropathic pain in which the fourth lumbar spinal nerve is transected (SpNT: spinal nerve transection) shows pain behavior that is resolved spontaneously. Recently, we found that a CD11c+ microglia subset emerged in the spinal cord after SpNT is necessary for the pain resolution. However, the role of CD11c+ microglia in other neuropathic pain models remains to be determined, especially in spared nerve injury (SNI) model that does not exhibit the spontaneous resolution of pain behavior. In this study, we found the number of CD11c+ microglia in the spinal cord was lower in the SNI model than the SpNT model, suggesting that prolonged behavioral pain hypersensitivity in the SNI model may be related to an impaired emergence of CD11c+ microglia. In addition, increasing the number of CD11c+ microglia by a cytokine administrated exogenously facilitated the resolution of pain behavior in both models. The alleviating effect was abolished by depletion of spinal CD11c+ microglia. Thus, increasing CD11c+ microglia or augmenting their function could be a new therapeutic strategy for neuropathic pain.

1-B-O01-5 一般演題（口頭）

# The involvement of spinal glial cell-derived lipocalin2 in the development of central post-stroke pain

Kazuo Nakamoto, Atsushi Ueda, Dan Tachibana, Shogo Tokuyama

*Dept. Clin. Pharm., Sch. Pharmaceu. Sci., Kobe Gakuin Univ.*

Central post-stroke pain (CPSP) is a type of central neuropathic pain, and its mechanisms remain unknown. Recently, we identified a significant increase of lipocalin 2 (LCN2) in the spinal cord of bilateral carotid artery occlusion (BCAO)-induced CPSP model mice using DNA microarray analysis. Generally, LCN2 is synthesized and secreted from activated glial cells. Also, glial cells derived LCN2 play a crucial role in the pathogenesis of neuropathic pain and stroke. In this study, we evaluated whether spinal glial cell-derived LCN2 is involved in the development of central post-stroke pain. Male ddY mice were subjected to 30 min of BCAO. Mechanical hypersensitivity was assessed by the von Frey test. LCN2 protein and its mRNA were evaluated by immunofluorescence stain and real-time PCR, respectively. We tested the expression of LCN2 in lipopolysaccharide (LPS)-activated MG6 microglial cells. BCAO mice showed hypersensitivity against mechanical stimuli and the activation of microglia and astrocyte in the spinal cord 3 days after BCAO. Spinal LCN2 protein was significantly increased and observed in the superficial dorsal horn of BCAO mice. LPS-activated microglial cells significantly and dose-dependently increased LCN2 mRNA expression. These results indicate that LCN2 in the spinal glial cells may involve in the development of CPSP.

1-B-O02-1 一般演題（口頭）

# Antioxidant mechanisms of hypotaurine by an action of taurine transporter (TauT) in hamster sperm

Takei Gen L.[1], Yasuhiro Horibata[2], Fubito Toyama[3], Keitaro Hayashi[1], Asuka Morita[1], Motoshi Ouchi[1,4], Tomoe Fujita[1]

*[1]Dept. Pharmacol. Toxicol., Dokkyo Medical Univ., [2]Dept. Biocheml., Dokkyo Medical Univ., [3]Sch. Engr., Utsunomiya Univ., [4]Grad. Sch. Nsg., Chiba Univ.*

Mammalian sperm, including human, must undergo several physiological and biochemical changes, collectively called capacitation, to be fertilization-competent. Capacitated sperm actively generate reactive oxygen species (ROS). A low level of ROS facilitates capacitation whereas an excessive ROS impairs capacitation. Hypotaurine (HT) is a precursor of taurine, and is abundant in the oviduct. HT is known to mitigate oxidative stress in hamster sperm, and is transported by taurine transporter (TauT) in a $Na^+$- and $Cl^-$- dependent manner. However, how HT protect sperm from oxidative stress remains unknown. This study aimed to elucidate the antioxidant mechanisms by HT in hamster sperm, focusing on the involvement of TauT.

We first examined the effects of HT on sperm motility, intra- and extra- cellular ROS levels. HT was shown to be necessary to maintain motility. HT lowered intracellular ROS levels, but had no effect on extracellular ROS levels at the concentration tested, although HT itself has an antioxidative capacity at higher concentrations. Incorporation and enrichment of HT in sperm were confirmed by LC-MS/MS analysis. Next, the involvement of TauT was investigated. TauT was present in hamster sperm. $\beta$-alanine, a blocker of TauT, inhibited HT transport, increased intracellular ROS levels and impaired sperm motility. Moreover, the elimination of $Na^+$ and $Cl^-$ inhibited HT transport, and increased intracellular ROS levels.

In conclusions, the results indicate that hamster sperm incorporate and concentrate HT via TauT to protect themselves from ROS.

1-B-O02-2 一般演題（口頭）

# Structural complexity and dynamics in GPCRs revealed by vibrational spectroscopy

Kota Katayama[1,2], Kohei Suzuki[1], Ryoji Suno[3], Hideki Kandori[1,2]

*[1]Nagoya Institute of Technology, [2]OptoBioTechnology Research Center, [3]Kansai Medical University*

GPCR signalling utilizes an allosteric coupling between the extracellular facing ligand-binding pocket and the cytoplasmic domain of the receptor that interacts with the signalling proteins. GPCR ligands impart different level of activation or deactivation of signalling proteins via GPCRs that are selectively and specifically regulated, in phenomena called ligand efficacy. The ligand efficacy remarkably affects the therapeutic properties of the ligand. Therefore, it is important to understand the molecular mechanism that determines the ligand efficacy in drug discovery research. Recently, we have attempted to use FTIR spectroscopy to study the conformational changes in muscarinic receptor ($M_2R$) induced by ligand binding. The systematic ligand binding-induced difference FTIR spectroscopy on ligands with four different efficacies (agonist, partial agonist, antagonist, and inverse agonist) demonstrate the novel direct method for the quantitative evaluation of ligand efficacy on $M_2R$. Notably, FTIR signals strongly correlates with the results of G-protein activity in cells. Thus, this approach emphasizes that the FTIR signal can serve as a probe to distinguish the ligand efficacy of $M_2R$, and how FTIR spectroscopy can efficiently contribute to elucidate the underlying mechanism of ligand engagement and action towards the receptors.

1-B-O02-3 一般演題（口頭）

# Regulatory mechanism of immediate early G protein-coupled receptor 3 gene expression during neuronal differentiation in PC12 cells

Shigeru Tanaka, Kouta Narai, Fumiaki Ikawa, Hiroko Shiraki, Kana Harada, Izumi Hide, Norio Sakai

*Hiroshima Univ. Grad. Sch. of Biomed & Health Sci., Dept. Mol. & Pharmacol. Neurosci.*

G protein-coupled receptor 3 (GPR3) is highly expressed in various neurons and can constitutively activate the G$\alpha$s protein in the absence of ligands, thereby elevating the basal intracellular cAMP levels. We have shown that GPR3 is upregulated during neuronal differentiation and contributes to neurite outgrowth and neuronal survival. Meanwhile, GPR3 is rapidly induced in neurons, T cells, and mast cells upon stimuli; however, the potential mechanisms related to the rapid GPR3 induction remain elusive. In this study, we investigated the regulatory mechanism underlying GPR3 expression and its effect on downstream gene expression during neuronal differentiation in PC12 cells. PC12 cells stimulated using the cAMP activator forskolin strongly upregulated GPR3 mRNA as early as 1–2 h, declining thereafter. In addition, the GPR3 expression induced by forskolin was significantly augmented with the increased intracellular $Ca^{2+}$ level produced by ionomycin. In addition, a luciferase-based promoter assay revealed that the cAMP response element in the 5'-flanking region of the rat GPR3 genome was associated with GPR3 transcription. Moreover, the upregulated GPR3 expression resulted in increased NR4A1 gene expression, further upregulating the expression of synapsin1, a downstream target of NR4A1. These results suggest that immediate early GPR3 upregulation by cAMP and $Ca^{2+}$ stimuli may further enhance cAMP signaling, thereby modulating downstream gene expressions.

1-B-O02-4 一般演題（口頭）

# NCX3 deficiency in the prefrontal cortex induces hyperactivity and social deficits via aberrant dopaminergic neurotransmission

Ryo Inagaki[1], Satomi Kita[2], Nozomu Niwa[1], Takahiro Iwamoto[3], Kohji Fukunaga[4], Shigeki Moriguchi[1]

*[1]Research Center for Pharmaceutical Development, Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai, Japan., [2]Department of Pharmacology, Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima, Japan,[3]Department of pharmacology, Faculty of Medicine, Fukuoka University, Fukuoka, Japan., [4]BRI Pharma Inc.*

$Na^+$/ $Ca^{2+}$ exchangers (NCXs) are predominantly expressed in neuronal plasma membranes and consist of three mammalian NCX isoforms (NCX1, NCX2, and NCX3). However, the biological function of NCX in the brain still remains unknown. Here, we examined behavioral changes, as well as underlying molecular properties in the NCX3 heterozygous ($NCX3^{+/-}$) mice. We found that hyperactivity and social deficits in $NCX3^{+/-}$ mice, which are ameliorated by the treatment of methylphenidate. In addition, we have identified that NCX3 was localized in the dopaminergic neuron of the ventral tegmental area which was the source of the dopamine innervation of the prefrontal cortex (PFC). In the PFC, $NCX3^{+/-}$ mice displayed decreased extracellular levels of dopamine triggered by social stimuli and persistent elevation of basal dopamine levels relative to WT mice. In concordance with the increase of extracellular dopamine levels in the PFC, $NCX3^{+/-}$ mice exhibited the activation of dopamine D1 receptor signaling pathways including PKA and DARPP-32 relative to WT mice in the PFC. Thus, the decreased expression of NCX3 leads to impair dopaminergic neurotransmission in the PFC, which likely accounts for the hyperactivity and social dysfunction in $NCX3^{+/-}$ mice.

1-B-O02-5 一般演題（口頭）

# Elucidation of the evolutionary conservation of the central serotonergic system in chicken by molecular dissection

Toshiyuki Fujita, Shinji Yamaguchi

*Teikyo Univ.*

The central serotonergic system is involved in various functions such as instinct, emotion, and cognitive functions in mammals. However, it is not clear how much molecular and functional conservation the central serotonergic system has in vertebrates. It has become clear that birds have advanced cognitive functions comparable to mammals. Therefore, we attempted to elucidate the molecular and anatomical structure of the central serotonergic system using the chicken (*Gallus gallus domesticus*) as an avian model.

First, we determined the location of the dorsal raphe (DR) nucleus and median raphe (MR) nucleus in the chicken using the expression distribution of the chicken orthologs of *serotonin transporter* (*SERT*) and *tryptophan hydroxylase 2* (*TPH2*) in the brainstem. Next, we clarified the serotonin receptors (5-HT receptors, 5-HTRs) expressed in serotonergic neurons contained in the DR and MR (Fujita, et al., 2022a). Our data indicate that the molecular properties of serotonergic neurons are evolutionarily well conserved between birds and mammals. In addition, we comprehensively elucidated the expression regions of almost all *5-HTR* genes in the chicken telencephalon (Fujita, et al., 2020; 2022b). Our data has comprehensively revealed the brain regions and receptors modulated by serotonin in the avian telencephalon, making it possible to access the understanding of the neural circuits that govern cognition and emotion modulated by the serotonergic system in birds.

1-B-O03-1 一般演題（口頭）

# Effects of a novel RyR2 specific inhibitor on arrhythmias in catecholaminergic polymorphic ventricular tachycardia (CPVT) model mice

Nagomi Kurebayashi[1], Masami Kodama[1,6], Takashi Murayama[1], Ryosuke Ishidaishida[2], Shuichi Mori[2], Masami Sugihara[3], Masato Konishi[1], Aya Miura[4], Hajime Nishio[4], Yukiko U. Inoue[5], Takayoshi Inoue[5], Satoru Noguchi[5], Kazuho Sakamoto[6], Junko Kurokawa[6], Hiroyuki Kagechika[2], Takashi Sakurai[1]

*[1]Dept Pharmacol, Juntendo Univ Sch Med, [2]Inst Biomat Bioeng, TMDU, [3]Dept Clin Lab Med, Juntendo Univ Sch Med, [4]Dept Leg Med, Hyogo Med Univ, [5]Natl Inst Neurosci, NCNP, [6]Dept Bio-Informational Pharmacol, Sch Pharmaceut Sci, Univ Shizuoka*

Gain-of-function mutations in RyR2 are known to cause lethal arrhythmias such as catecholaminergic ventricular tachycardia (CPVT). In CPVT, reduction of RyR2 activity is thought to suppress arrhythmias, but there are no clinically available antiarrhythmic drugs with RyR2-specific inhibitory action. We developed a high-affinity (IC50 of ~15nM) and selective RyR2 inhibitor, TMDJ-035, based on a hit compound identified in a high-throughput screening. TMDJ-035 effectively suppressed arrhythmias in CPVT mouse models harboring mutant RyR2s. Unlike conventional anti-arrhythmic drugs, i.e., Na channel inhibitors, Ca channel inhibitors, ß-blockers, TMDJ-035 did not affect ECG parameters or cardiac contractile function at the effective doses. Our results demonstrate that the specific suppression of RyR2 activity is highly effective in preventing and treating arrhythmias caused by RyR2 hyperactivation.

1-B-O03-2 一般演題（口頭）

# Excitation-transcription coupling in smooth muscle is associated with vascular remodeling

Yoshiaki Suzuki[1], Masatake Araki[2], Kimi Araki[2], Gerald Zamponi[3], Wayne Giles[3], Yuji Imaizumi[1], Hisao Yamamura[1]

*[1]Dept. Mol & Cell. Pharmacol., Grad. Sch. Pharmaceut. Sci., Nagoya City Univ., [2]Institute of Resource Development and Analysis, Kumamoto University, [3]Cumming School of Medicine, University of Calgary*

Various stresses loaded on the arteries induce vascular remodeling. Macrophages accumulated in the vascular wall promote dedifferentiation and proliferation of smooth muscle cells (SMC) and induce vascular remodeling. However, it remains unclear how arteries sense various stresses and accumulate macrophages. We found that $Ca^{2+}$ signals in SMCs are converted into gene transcription via excitation-transcription (E-T) coupling, which recruits macrophages to the vascular wall. Imaging analyses revealed that the activation of a complex consisting of voltage-dependent $Ca^{2+}$ channel (Cav1.2), $Ca^{2+}$/CaM dependent kinase kinase (CaMKK)-2, and CaMK1$\alpha$ formed in caveolae induces transcription of genes, such as chemokines, cytokines, and leukocyte adhesion molecules. When pressure overload was applied to mouse mesenteric arteries *in vivo*, migration of macrophages to the vascular adventitia and medial hypertrophy were detected. These changes were attenuated in deletion of caveolin-1 or CaMKK2 genes as well as the administration of a CaMKK2 inhibitor. These data suggest that the sustained increase in intracellular $Ca^{2+}$ level due to mechanical stress is converted into the transcription of proinflammatory genes through E-T coupling, which results in the accumulation of macrophages and subsequent inflammation causes vascular remodeling.

1-B-O03-3 一般演題（口頭）

# Molecular dynamics simulation study for the molecular instability of NBD1 of CFTR induced by disease-associated mutations causing Cystic Fibrosis in Japanese patients.

Hikaru Sohma[1], Rio Kimishima[1], Tadaomi Furuta[2], Yoshiro Sohma[1]

*[1]Div Mol Ther, Grad Sch Pharm, IUHW, Ohtawara, Japan,[2]Sch Life Sci Tech, Tokyo Inst Tech, Yokohama, Japan*

Cystic fibrosis (CF) is a fatal genetic disorder caused by abnormal function of the cystic fibrosis membrane conductance regulator (CFTR). CFTR is an ATP-binding cassette (ABC) transporter expressed in the transport epithelia and plays a central role in the membrane transport system. Therefore, CFTR is involved in many respiratory and digestive diseases, including CF, chronic obstruction (COPD) and chronic pancreatitis.

CF is common in Caucasians. Deletion of phenylalanine at position 508 (ΔF508) in Nucleotide Binding Domain 1 (NBD1) is the most common CF-associated mutation and causes defects in CFTR trafficking to the plasma membrane (class II). Several Japanese-specific CF-causing mutations have been also identified in NBD1. The NBD1 molecular instability should lead to the CFTR protein degradation causing the class II trafficking defect.

In this work, using molecular dynamics simulations, we investigated the molecular fluctuation of NBD1 with and without the disease-associated mutations and discuss about the pathophysiological mechanisms of the Japanese CF.

1-B-O03-4 一般演題（口頭）

# Expression of Smoc2, a matricellular protein, in the cochlea of the mammalian inner ear

Ono Kazuya[1], Takeru Ota[1], Yoshifumi Takahata[2], Riko Nishimura[2], Hiroshi Hibino[1]

*[1]Div. Glocal. Pharmacol., Dept. Pharmacol. Grad. Sch. Med. Osaka Univ.,[2]Dept. Mol. Cell. Biochem. Grad. Sch. Dent. Osaka Univ.*

Cochlea of the inner ear is essential for hearing. Cochlear sensory epithelium consists of hair cells, supporting cells, and tectorial membrane (TM), an extracellular matrix lying over the apical surface of the epithelium. Acoustic stimuli vibrate the epithelium and deflect hair bundle of the hair cells. This event opens mechanosensitive channels on hair bundle, which induces cation entry and excites the hair cells. TM is crucial for vibration of hair bundle. Although sensory differentiation and maturation occurs rapidly around birth, the regulatory factors remain obscure. Here, by comparing bulk RNA transcripts of the epithelium between neonatal (1 day old) and adult (3 month old) mice, we found that Smoc2 (SPARC-related modular calcium binding 2) encoding a matricellular protein is highly expressed during development. In neonatal mouse cochlea, Smoc2 mRNA was detected in hair cells as well as a subpopulation of supporting cells, which produces TM components. Immunolabeling revealed that, besides plasma membranes of these cell types, Smoc2 protein could be also localized to TM. Because Smoc2 can interact with BMP signaling that is required for hair cell formation (Ohyama et al., JN 2010), this matricellular protein may regulate the development of not only TM but also sensory epithelium.

**1-B-O03-5** 一般演題（口頭）

# Physiological and pharmacological properties of a novel $H^+$,$K^+$-ATPase ATP13A2 in neuronal lysosomes

Takuto Fujii[1], Shushi Nagamori[2], Pattama Wiriyasermkul[2], Takahiro Shimizu[1], Yoshiaki Tabuchi[3], Tomoyuki Okumura[4], Tsutomu Fujii[4], Hiroshi Takeshima[5], Hideki Sakai[1]

*[1]Dept. Pharmaceut. Physiol., Fac. Pharmaceut. Sci., Univ. Toyama.,[2]Ctr. SI Med. Res., Jikei Univ. Sch. Med.,[3]Div. Mol. Gen. Res., Life Sci. Res. Ctl., Univ. Toyama,[4]Dept. Surg. Sci., Fac. Med., Univ. Toyama,[5]Dept. Biol. Chem., Grad. Pharmaceut. Sci., Kyoto Univ.*

ATP13A2 (PARK9), a P5-type ATPase, is associated with autosomal recessive early-onset Parkinson's disease (PD), known as Kufor-Rakeb syndrome. In the present study, we found that ATP13A2 functions as a novel lysosomal $H^+$,$K^+$-ATPase that transports $K^+$ from the lysosomal lumen to the cytoplasm and $H^+$ from the cytoplasm to the lysosomal lumen. Interestingly, ATP13A2 has unique pharmacological properties: its $K^+$-ATPase activity was significantly inhibited by $K^+$-competitive acid blockers (P-CABs) vonoprazan ($IC_{50}$ = 0.8 $\mu$M) and SCH28080 ($IC_{50}$ = 1.2 $\mu$M), and a vacuolar $H^+$-ATPase inhibitor bafilomycin A1 ($IC_{50}$ = 0.5 nM). On the other hand, a proton pump inhibitor (PPI) omeprazole and a $Na^+$,$K^+$-ATPase inhibitor ouabain did not affect the ATPase activity. The inhibition of ATP13A2 activity by P-CABs caused lysosomal alkalinization and $\alpha$-synuclein accumulation, which are pathological hallmarks of PD, in human neuronal SH-SY5Y cells. In addition, PD-associated mutations of ATP13A2 markedly reduced the expression and $K^+$-transporting activity of the ATPase. These results suggest that the $H^+$/$K^+$-transporting function of ATP13A2 contributes to acidification and $\alpha$-synuclein degradation in the lysosomes of neurons.

2-B-O04-1 一般演題（口頭）

# The potential effects of single dose of melatonin on learning and memory function

Sano Masahiro, Kohji Fukunaga, Ichiro Kawahata

*Dept. of CNS drug innov., Grad. Sch. of Pharmaceut. Sci., Tohoku Univ.*

Melatonin, primarily synthesized and secreted by the pineal gland, exerts multifaceted roles in reducing oxidative stress, regulating circadian rhythms, modulating immune functions, and ameliorating age-related cognitive impairments in mice through chronic administration. Recent research has demonstrated that a single melatonin dose can enhance learning and memory in mice. However, the precise mechanisms underlying melatonin's cognitive-enhancing properties remain elusive. In our study, we administered melatonin intraperitoneally, along with its MT1/MT2 receptor agonist, ramelteon. This treatment notably improved long-term memory performance in mice, as assessed through the Object Recognition Test (ORT). Western blot analysis revealed that phospho-CaMKII$\alpha$ levels decreased in the hippocampus across all drug administration groups but increased in the lateral cortex in response to all treatments. Phospho-CaMKII$\beta$ exhibited a decrease in the hippocampus within the ramelteon group and an increase in the lateral cortex within the melatonin groups. Additionally, phospho-ERK levels increased in the hippocampus within the ramelteon group and in the lateral cortex across all treatment groups after 30 minutes. Furthermore, phospho-CaMKII$\beta$ decreased in the hippocampus across all drug administration groups after 2 hours. In the medial prefrontal cortex, we observed a decrease in phospho-CaMKII$\beta$ levels within the melatonin group after 30 minutes, and an increase in phospho-ERK levels within the same group after 2 hours. Alongside these findings, we conducted a Y-maze test and observed that melatonin administration significantly enhanced working memory in mice. This indicates that a single melatonin dose may have a broad spectrum of memory-enhancing effects.

2-B-O04-2 一般演題（口頭）

# PKB/AKt pathway involves in facilitation of inhibitory synaptic transmission by insulin in the insular cortex

Yuka Nakaya[1], Satomi Kobayashi[1,2], Kouhei Kitano[1], Masayuki Kobayashi[1]

*[1]Dept. Pharmacol. Nihon Univ. Sch Dent., [2]Dept. Biol. Nihon Univ. Sch Dent.*

Insulin receptors are expressed in the cerebral cortex including the insular cortex (IC). However, little information is available for the mechanisms how insulin modulates neural activities in the cerebral cortex. Here, we examined effects of insulin on synaptic transmission between fast-spiking GABAergic neurons (FSNs) and pyramidal neurons (PyNs) in the IC using multiple whole-cell patch-clamp recording.

We found that insulin increased the repetitive spike firing rate with a decrease in the threshold potential without changing the resting membrane potentials and input resistance of FSNs. The amplitude of unitary IPSCs (uIPSCs) was significantly increased and paired-pulse ratio of uIPSCs was significantly decreased by insulin. S961, an insulin receptor antagonist, and lavendustin A, a tyrosine kinase inhibitor, interrupted the effect of insulin on uIPSCs. In addition, wortmannin, a PI-3 kinase inhibitor, deguelin, a PKB/Akt inhibitor, and Akt inhibitor VIII significantly suppressed insulin-induced facilitation of uIPSCs. On the other hand, the effect of insulin was not blocked by PD98059, a MAPK inhibitor.

These results suggest that insulin increased release of GABA from FSNs by activating PKB/Akt cascade.

2-B-O04-3 一般演題（口頭）

# Single-molecule imaging within brain tissue

Yohei Okubo[1], Shigeyuki Namiki[2], Daisuke Asanuma[2], Takashi Sakurai[1], Kenzo Hirose[2]

*[1]Dept. Pharmacol., Fac. Med., Juntendo Univ., [2]Dept. Pharmacol., Grad. Sch. Med., Univ. Tokyo*

Single-molecule imaging, a super-resolution live imaging method, has enabled direct tracking of nanoscale movements of individual proteins in living cells. However, its application has been limited to dissociated cell cultures due to technical constraints, which hindered the investigation of individual protein behavior within intact tissues. To overcome this limitation, we aimed to develop a method for single-molecule imaging within tissue specimens. We introduced a novel chemical tag technology named De-QODE, consisting of a small-molecular QODE probe and DeQODE protein tag. The QODE probe, initially non-fluorescent, becomes highly fluorescent upon reversible binding to the DeQODE tag. These unique properties allow for fluorescent labeling of proteins of interest with remarkably low-background fluorescence even within tissue samples. By harnessing De-QODE-based single-molecule imaging, we successfully achieved high-density tracking of synaptic molecules in neurons within acutely isolated brain slices. This groundbreaking approach provides unprecedented insights into the dynamic behavior of proteins within the intricate tissue environment, significantly advancing our understanding of cellular processes in their natural context.

**2-B-O04-4** 一般演題（口頭）

# Abnormal shortening of hippocampal telomere in an animal model of depression

Hiroki Shikanai[1,2], Tsugumi Shindo[1], Kazune Ozaki[1], Atsuko Ohashi[1], Ikuo Otsuka[3], Akitoyo Hishimoto[3], Takeshi Izumi[1,2]

*[1]Dept. Pharmacol., Fac. Pharmaceut. Sci., Health Sci. Univ. Hokkaido, [2]Adv. Res. Promotion Ctr., Health Sci. Univ. Hokkaido, [3]Dept. Psychiatry, Kobe Univ. Grad., Sch., Medicine*

Telomere length is one of the key components of cell lifespan and is maintained by the telomerase reverse transcriptase (TERT), an elongation enzyme for telomere. Increased TERT activity has been reported in cancer cells, while abnormal shortening of telomere length has been reported in several diseases such as diabetes and hypertension. We also reported an abnormal shortening of telomere length in the blood and brain of suicide victims. In this study, we investigated the relationship between depression and brain telomeres using an animal model of depression. We evaluated adult rats exposed to juvenile stress as an animal model of depression. Model rats showed prolonged immobility time in the forced swimming test. Abnormal shortening of telomere length was observed in the medial prefrontal cortex and dorsal hippocampus of model rats. TERT was significantly decreased in the hippocampus of model rats. Shortening of telomere length and decrease of TERT in the hippocampus of model rats were restored by repeated administration of escitalopram. Furthermore, we found abnormal signaling of GSK3$\beta$ and $\beta$-catenin, which are depression-related genes and transcriptional regulators of TERT, in the hippocampus of model rats. These results provide novel pathophysiological insights into depression.

**2-B-O04-5** 一般演題（口頭）

# Induction of LTP of inhibitory synapses from parvalbumin-immunopositive neurons to pyramidal neurons using optogenetics

Kobayashi Satomi[1,2], Satoshi Fujita[2], Masayuki Kobayashi[1]

*[1]Dept. Pharmacol., Nihon Univ. Sch. Dent., [2]Dept. Biol., Nihon Univ. Sch. Dent.*

Repetitive nociception induces ectopic pain in the orofacial area by changing local neural circuits in the insular cortex (IC). Parvalbumin-immunopositive neurons (PVNs) send abundant projections to pyramidal neurons (PNs) and strongly suppress PN activities. Therefore, potentiation of PVNs→PNs inhibitory synaptic connections in the IC may suppress ectopic pain induction. In the present study, we investigated how repetitive stimuli by optogenetics applied to PVNs change the efficiency of synaptic connections from PVNs to PNs.

First, we selectively expressed ChR2 in PVNs using Cre-loxP systems. Application of blue light to PVNs in the IC slice preparation induced action potentials in PVNs and inhibitory postsynaptic currents (IPSCs) in PNs. We investigated the protocol inducing long-term potentiation (LTP) of IPSCs. We found that optical stimuli similar to the theta burst stimulation increased to 142% of the amplitude of IPSCs. This LTP induction was independent from glutamate receptor activation. In the future, we apply this protocol to *in vivo* preparation to treat model animals with ectopic pain.

2-B-O05-1 一般演題（口頭）

# Galectin-1-elicited axonal regeneration in the brains and memory recovery effects in Alzheimer‘s disease model mice

Yang Ximeng, Chihiro Tohda

*Section of Neuromedical Science, Inst. of Natural Med., Univ. of Toyama*

Alzheimer‘s disease (AD) is a neurodegenerative disorder characterized by A$\beta$ deposition and neural networks disruption in the brain. We previously found that diosgenin, a constituent of Dioscorea Rhizoma, promoted long-distance axonal regeneration in the brains and recovered memory deficits in an AD model (5XFAD) mouse. In the present study, we aimed to clarify molecular mechanisms for accurate pathfinding of degenerated axons in AD brains.
Axon-regenerated neurons (after diosgenin administration) in the neural circuits contributing memory formation; from the hippocampus to the prefrontal cortex, were visualized by retrograde tracings. Naïve neurons and axon-regenerated neurons in the brain slices were individually captured by laser microdissection, and Galectin-1 was identified as a drastically upregulated molecule in axon-regenerated neurons by DNA microarray. Overexpression of Gelectin-1 in hippocampal neurons promoted axonal regeneration and recovered memory deficits in 5XFAD mice. Galectin-1 expressed on the membranes of growth cones and was attracted by extracellular Secernin-1 to promote axonal guidance from the hippocampus to the prefrontal cortex.
Our study identified a novel axonal guidance pair, Galectin-1 and Secernin-1, for axonal regeneration in AD brains, which is a promising novel therapeutic strategy for AD.

**2-B-O05-2** 一般演題（口頭）

# Mechanism of necrotic tissue drainage after cerebral ischemia

Toshinori Sawano[1], Haiyang Sun[1], Momoka Okada[1], Jin Nakatani[1], Shinobu Inagaki[2,3], Takayuki Nakagomi[4,5], Tomohiro Matsuyama[5], Hidekazu Tanaka[1]

*[1]Pharmacol. Lab., Dept. Biomed. Sci., Ritsumeikan Univ., [2]Dept. Child Dev. and Mol. Brain. Sci., United Grad. Sch. Child Dev., Osaka Univ., [3]Dept. PT., Yukioka Col. Health Sci., [4]Lab. Neurogenesis and CNS Repair., Inst. Adv. Med. Sci., Hyogo Med. Univ., [5]Dept. Therap. Prog. Brain Dis., Hyogo Med. Univ.*

Ischemic stroke leads to liquefactive necrosis, which disappears from intracranial space with the passage of time. The necrotic tissue contains various types of neurotoxic components. Thus, effective clearance of necrotic debris is important for non-necrotic areas to maintain homeostasis. However, the precise mechanism of necrotic tissue drainage is not revealed. Currently, we found that endothelial cells proliferation was significantly upregulated in the necrotic area at 3 days after middle cerebral artery occlusion (MCAO). Concomitantly, microglia were induced in the necrotic area. Microglial depletion by PLX3397 administration significantly inhibited the MCAO-induced endothelial cells proliferation. Furthermore, disappearance of necrotic tissue was significantly inhibited by microglial depletion. These results suggest that the necrotic tissue is drained through the vessels in the necrotic tissue, which is provided by microglia.

**2-B-O05-3** 一般演題（口頭）

# *P.gingivalis* promotes influx of amyloid beta into the brain via cerebrovascular endothelial cells

Zhou Wu[1,2], Fan Zeng[3], Shuge Gui[4], Shinsuke Mizutani[2,5], Haruhiko Kashiwazaki[5]

*[1]Dept. Cell Biol., Aging Sci. Pharmacol., Kyushu Univ., [2]OBT Research Center, Kyushu Univ., [3]Shenzhen Key Lab. Immuno. Neurol. Dis., Shenzhen Inst. Adv. Tech., Acad. Sci. CHN, [4]Dept. Dept. Oral Maxillofac. Surg. Kyushu Univ., [5]Dept. Geriatric Dentistry and Perioperative Medicine in Dentistry, Kyushu Univ.*

[Background and purpose] Cerebral amyloid angiopathy (CAA) is observed in more than 80% of Alzheimer's disease (AD) patients. Receptor for advanced glycation end products (RAGE) mediates amyloid β (Aβ) influx into brain. In this study, we test our hypothesis that P.g promotes Aβ influx. [Methods and Results] In hCMEC/D3 cells, RAGE expression was significantly increased in the P.g-infected hCMEC/D3 cells in compassion to that in uninfected cells. The P.g-increased RAGE expression in hCMEC/D3 cells was inhibited by pretreating with NFκB inhibitor. In addition, cathepsin B (CatB) was increased in the P.g-infected hCMEC/D3 cells, and CatB specific inhibitor suppressed both IκBα degradation and RAGE expression in the P.g-infected hCMEC/D3 cells. Using fluorescently labeled $A\beta_{1\text{-}42}$, the amount of $A\beta_{1\text{-}42}$ in basolateral medium of the P.g-infected hCMEC/D3 cells was 16-fold higher than that in uninfected cells. In 15-month old mice, RAGE expression in CD31-positive cerebral endothelial cells was increased 2-fold in the P.g-infected mice in compassion to that in PBS-injected mice. Furthermore, $A\beta_{1\text{-}42}$ expression which surrounding the CD31-positive cerebral endothelial cells was increased 11-fold in in compassion to that in PBS-injected mice. Moreover, the memory function was decreased in the P.g-infected mice. [Conclusion] These observations demonstrate that up-regulated RAGE expression in cerebral endothelial cells mediates the Aβ influx after *P. gingivalis* infection, and CatB plays a critical role in regulating the NF-κB/RAGE expression.

2-B-O05-4 一般演題（口頭）

# Characterization of aripiprazol on motor symptoms in mouse models of Parkinson‘s disease

Jiro Kasahara, Mihiro Sakashita, Hiten Iwamoto, Masatoshi Ogawa

*Dept. Neurobiol. Theapeu., Grad. Sch. Biomed. Sci., Tokushima Univ.*

Aripiprazol (APZ) is a partial agonist of dopamine D2 receptor, which is widely used to treat psychiatric diseases such as schizophrenia. Because its agonistic activity is weaker than intrinsic dopamine, both an inhibitory effect in excess dopamine and a stimulatory effect in dopamine depletion are expected. Lower expression of extrapyramidal syndrome is also expected because of its agonistic effect on 5-HT2A receptor, however the relationship of APZ to motor symptoms are not well characterized yet. Here we report the effect of APZ on motor deficits in two mouse models of Parkinson‘s disease (PD). In acutely and peripherally MPTP-intoxicated mice, APZ normalized both a prolonged duration of akinesia-like immobility in bar test and an increased walking time in beam-walking test. In hemi-PD mice injected with 6-OHDA into the right medial forebrain bundle, APZ improved motor deficits characterized by a spontaneously circling activity and an asymmetrical step of hind limbs. Moreover, APZ showed a significantly lower score of abnormal involuntary movements, which is an index of drug-induced dyskinesia, compared to levodopa when administered 21 days. From the aspect of low motor adverse effects, these results may provide rationale for the use of APZ for psychosis in PD patients, although careful clinical observation is still required.

**2-B-O05-5** 一般演題（口頭）

# Role of the hypothalamus and the nucleus accumbens in regulation of glucose metabolism by dopamine $D_2$ receptors

Hiroko Ikeda, Naomi Yonemochi

*Dept Pathophysiol Therap, Hoshi Univ Sch Pharm Pharmaceut Sci*

The central nervous system might regulate glucose homeostasis, but its mechanism is unclear. We investigated the role of central dopamine $D_2$ receptors in glucose homeostasis. I.c.v. injection of both dopamine $D_2$ receptor agonist quinpirole and antagonist l-sulpiride increased plasma glucose levels. Hyperglycemia induced by quinpirole or l-sulpiride was diminished following fasting which decreases hepatic glycogen level, and these drugs did not affect hyperglycemia in the pyruvate tolerance test. Injection of $\beta_2$ adrenoceptor antagonist ICI 118,551, which blocks input from the sympathetic nerves to the liver, inhibited hyperglycemia induced by l-sulpiride, but not quinpirole, whereas hyperglycemia induced by quinpirole, but not l-sulpiride, was inhibited by hepatic vagotomy which blocks input from parasympathetic nerves. In addition, injection of quinpirole into the hypothalamus or nucleus accumbens increased plasma glucose levels, which was blocked by l-sulpiride. Taken together, it is suggested that stimulation of central dopamine $D_2$ receptors including the hypothalamus and the nucleus accumbens causes hyperglycemia by increasing hepatic glycogenolysis through parasympathetic nerves whereas blockade of central dopamine $D_2$ receptors causes hyperglycemia by increasing hepatic glycogenolysis through parasympathetic nerves.

2-B-O06-1 一般演題（口頭）

# Effects of physical reaction on emotional state: development of optogenetic-based cardiac pacing in awake freely moving mice

Yamashita Akira[1], Jun Kaminosono[2], Yuki Kambe[3], Satoshi Imai[1], Akihide Tanimoto[4], Tomoyuki Kuwaki[2]

*[1]Dept. Medical Neuropharmacol., Wakayama Med. Univ., Sch. Pharmaceut. Sci.,[2]Dept. Physiol1., Kagoshima Univ., Grad. Sch. Med. & Dent. Sci., [3]Dept. Pharmacol. Kagoshima Univ., Grad. Sch. Med. & Dent. Sci., [4]Dept. Pathol. Kagoshima Univ., Grad. Sch. Med. & Dent. Sci.*

Emotion affects physical reaction by the autonomic nervous system in the top-down process. However, there are few reported whether physical reactions, such as increased heart rate, might induce emotional changes like anxiety or fear responses. Here, we have investigated emotional behavior by controlling heart rate directly by a drug administration or an optogenetic-based cardiac pacing system in freely moving mice. First, the heart rate was controlled by ivabradine, an inhibitor of the hyperpolarization-activated cyclic nucleotide-gated channels. We found that ivabradine evoked bradycardia and partially reduced anxiety-like behavior. Second, heart rate was controlled by the optical cardiac pacing in freely moving mice. In many previous studies, the pacing was applied ex vivo or in anesthetized animals. Therefore, we developed the optical cardiac pacing system in awake, freely moving mice and simultaneously measured electrocardiograms. We found that Optically increased heart rate by using this system potentially enhanced anxiety-like behavior. These results suggested that emotional states are partially driven by heart rate in the bottom-up process. In conclusion, the brain function and the feedback from the physical reactions must be considered together to understand the mechanisms of expressed emotion.

**2-B-O06-2** 一般演題（口頭）

# Real-time intraocular antiglaucoma drugs measurement in porcine eyes using boron-doped diamond microelectrodes.

Genki Ogata[1], Mao Yoneda[1], Risa Ogawa[1], Ai Hanawa[1], Kai Asai[1], Reiko Yamagishi[2], Megumi Honjo[2], Makoto Aihara[2], Yasuaki Einaga[1]

*[1]Dept. of Chem., Fac. of Sc.i and Tech., Keio Univ.,[2]Dept. of Ophthalmol., Univ. of Tokyo Sch. Med.*

The primary treatment for glaucoma, the leading cause of intermediate vision impairment, involves administering ocular hypotensive drugs in topical eye drops. Observation of the real-time changes in the drugs through the cornea and reaching the anterior chamber is essential to improve or develop a safe, reliable, and effective medical treatment. Conventional methods such as LC-MS/MS are used to measure the temporal changes in the drug in the aqueous humor; however, this technique involves multiple measurements of the eyes of multiple test subjects to measure changes over time with high temporal resolution. To resolve this problem, we develop a measurement method that utilizes boron-doped diamond (BDD) microelectrodes to track the real-time drug concentrations in the anterior chamber of the eye. First, we optimize the method for continuously measuring timolol maleate (TIM), a sympathetic beta-receptor antagonist, and obtain the calibration curves of each BDD microelectrode in the aqueous humor collected from porcine eyes. Next, we demonstrate the continuous *ex vivo* monitoring of the TIM in the enucleated porcine eyes. The results suggest that changes in the intracameral TIM concentration can be monitored using BDD microelectrodes.

2-B-O06-3 一般演題（口頭）

# Wnt5a, produced by physiological mechanical stimulation on the periodontal ligament, regulates neuronal differentiation in the trigeminal mesencephalic nucleus

Kaori Takahashi[1], Takashi Yoshida[1,2], Takashi Nakamura[1], Minoru Wakamori[1]

*[1]Div. Mol. Pharmacol. Cell Biophys., Grad Sch. Dent., Tohoku Univ.,[2]Div. Pharmacol. Fac. Pharmaceut. Sci, Teikyo Heisei Univ.*

Although many cohort studies have reported an association between poor oral function and the development of dementia, the detailed molecular physiological mechanisms linking them are still unclear. When occlusal pressure is applied to the teeth, primary sensations are transmitted from the Ruffini endings in the periodontal ligament (PDL) to the trigeminal ganglion (TG) and the trigeminal mesencephalic nucleus (Me5), and from the muscle spindles of the masseter muscle to the Me5. Primary sensory neurons are usually located outside the brain. However, the Me5 is exceptionally located inside the brainstem. Recently, we have reported that the rat PDL (rPDL) cells express *NGF*, *BDNF*, *NT-4*, and *Wnt5a* mRNA, but only *Wnt5a* mRNA in rPDL cells increases by mechanical stimulation. We hypothesize that factors released from mechanically stimulated PDL cells regulate the maintenance of the Me5 neurons. The rPDL cells, which we established from rat molar teeth, were loaded with periodic mechanical stimulation (0.5 Hz, 15% elongation). The culture medium for the primary mouse Me5 neurons was replaced with the supernatant media of the rPDL cells with or without mechanical stimulation. The supernatant medium of the mechanically stimulated rPDL cells enhanced the neurite outgrowth and this effect was suppressed by anti-Wnt5a antibody. *In vivo*, Wnt5a was significantly decreased in the Me5 after tooth extraction by ELISA. These results suggest that Wnt5a, produced by physiological mechanical stimulation on PDL, regulates the outgrowth of Me5 neurons.

2-B-O06-4 一般演題（口頭）

# Administration of bisphosphonates adversely affect femoral heads in mild type hypophosphatasia model mice

Aki Takahashi[1], Kengo Hirai[2], Satoshi Ishiduka[1], Norio Kasahara[3], Satoru Matsunaga[4], Shinichi Abe[4], Seikou Shintani[2], Masataka Kasahara[1]

*[1]Dept. Pharmacol. Tokyo Dent. Coll., [2]Dept. Pediatric Dent. Tokyo Dent. Coll., [3]Dept. Histology and Dept. Biol. Tokyo Dent. Coll., [4]Dept. Anatomy. Tokyo Dent. Coll.*

Hypophosphatasia (HPP) is one of the congenital bone systems diseases in which mutations in the tissue-nonspecific alkaline phosphatase gene. It is classified into six types, perinatal severe, prenatal benign, infantile, childhood, adult, and odonto type HPP. HPP with femoral hypoplasia on fetal echography and premature tooth loss is easy to diagnose. In contrast, childhood over 4 years of age, adult, and odonto type HPP are not diagnostic of HPP, because they do not have typical symptoms. As a result, HPP cannot be diagnosed, so it is considered to be general osteoporosis or periodontal disease. The purpose of this study was to analyze the effects of administration of BP preparations on the femur of *Akp2*$^{+/-}$ mice, which are mild HPP model mice.

Zoledronic acid (Zol) was subcutaneously administered at 1 mg/kg (vol: 200 μL) every other week for a total of 5 times to 4-week-old *Akp2*$^{+/-}$ mice. One week later, the femurs were sampled and HE staining was performed for micro-CT analysis and histological analysis. As a control, *Akp2*$^{+/+}$ mice of the same age were used.

In the femoral head, degeneration of cartilage, invasion and destruction of fibers were confirmed only in *Akp2*$^{+/-}$ mice after administration of Zol. Activity analysis result, only the Zol-administered *Akp2*$^{+/-}$ mice showed lower values than the other mice in all items.

Our results suggest that administration of Zol formulations to patients with mild HPP may increase the risk of exacerbation of symptoms compared to healthy subjects. Consequently, it is important to accurately diagnose mild HPP patients.

**2-B-O06-5** 一般演題（口頭）

# Antioxidative activity of a novel antioxidant “Substance X” as a direct free radical scavenger

Tokumaru Osamu[1], Akihiro Higuchi[2], Takayuki Kawashima[3], Kazue Ogata[1], Kazuhiro Ueno[3], Shinji Miyamoto[3]

*[1]Dept. Physiol., Fac. Welfare Health Sci., Oita Univ.,[2]Front. Sci. Soc. Co-creat. Init, Kanazawa Univ.,[3]Dept. Cardiovasc. Surg., Oita Univ. Fac. Med.*

Purpose

We happened to synthesize by chance a novel drug called “Substance X” with antioxidative activity during an attempt to synthesize a known other drug. Here we report the dose-dependent free radical scavenging activity of Substance X against multiple free radicals.

Methods

Free radical scavenging activity of Substance X was evaluated against following nine species of free radicals by electron spin resonance spectroscopy with the spin-trapping method: hydroxyl radical, superoxide anion, *tert*-butyl peroxyl radical,*tert*-butoxyl radical, ascorbyl free radical, singlet oxygen, nitric oxide, DPPH and tyrosyl radical. By fitting sigmoid dose-response curves, reaction rate constants of Substance X with free radicals studied were estimated. Antioxidative activity against tissue lipids was assessed by TBARS assay.

Results

Substance X significantly scavenged all the free radicals examined in dose-dependent manners. The reaction rate constants of Substance X with some species of free radicals were significantly larger than those of edaravone. The inhibition of lipid oxidation by Substance X was comparable to that by edaravone.

Conclusions

Substance X dose-dependently scavenged multiple free radicals with reaction rate constants comparable to those of edaravone. It is speculated that the direct free radical scavenging activity might contribute to the antioxidative activity of Substance X. Patent pending.

2-B-O07-1 一般演題（口頭）

# Establishment of an Age-Related Hearing Loss *in vitro* Model Using Mouse Cochlear Cell Line HEI-OC1 Cells and Evaluation System for Autophagy pathway.

Iwai Yoshito, Oguro Yuji, Yasuda Shuta, Ueno Takafumi, Yoshida Shohei, Furuya Yuriko, Yamamoto Keiji

*Nagahama Institute for biochemical science, Oriental Yeast Co., ltd.*

[Background and Purpose] Age-related hearing loss (HL), which is caused by age-related disorders in inner ear tissues, has been a social problem because there is no clinical medicine for HL and HL in middle age is the most severe risk factor for dementia. Recent studies have reported that oxidative stress is deeply involved in disorders of inner ear cells and that suppressive effect on oxidative stress in the inner ear may delay the progression of age-related HL. In our study, we established an age-related HL *in vitro* model that hydrogen peroxide was treated with HEI-OC1 cells, a mouse cochlear cell line, and evaluated its effects on the autophagy mechanism.

[Methods] HEI-OC1 cells were cultured at 33℃ and 10% CO2. The expression of hair cell, supporting cell, and Spiral ganglion cell marker genes (*Myo7a*, *Gjb2*, *Bdnf*) in HEI-OC1 cells was analyzed by qPCR. HEI-OC1 cells treated with hydrogen peroxide at concentrations of 0.5 to 2 mM were measured LC3 protein levels and lysosomal activity, key markers of autophagy. In addition, 5 mM N-acetyl-L-cysteine (NAC), which has antioxidant effect, was applied HEI-OC1 cells with oxidative stress and was measured level of LC3 and lysosomal activity.

[Results] HEI-OC1 cells expressed specific inner ear marker genes such as *Myo7a*, *Gjb2*, and *Bdnf*. Hydrogen peroxide increased the level of LC3 protein of HEI-OC1 cells and decreased lysosomal activity of them in a dose dependent manner. Moreover, NAC ameliorated these impairments caused by oxidative stress significantly.

[Conclusion] HEI-OC1 cells were a undifferentiated inner ear cell line expressing various specific marker genes. We established an age-related HL *in vitro* model by exposure to hydrogen peroxide to them and an assay system using autophagy lysosomes pathway. The impairment of autophagy lysosome pathway by oxidative stress was ameliorated in treatment with NAC. Our data suggested that this system is used for screening drugs having strong antioxidant effects.

2-B-O07-2 一般演題（口頭）

# Dispensable role of Rac1 and Rac3 after cochlear hair cell specification

Hiroaki Mohri[1,2,3], Takashi Nakamura[1,2], Hirofumi Sakaguchi[2], Yuzuru Ninoyu[1], Naoaki Saito[1], Takehiko Ueyama[1]

*[1]Lab. of Mol. Pharmacol., Biosignal Research Center, Kobe Univ.,[2]Dept. of Otolaryngology-Head and Neck Surgery, Kyoto Prefectural Univ. of Med.,[3]Dept. of Otolaryngology-Head and Neck Surgery, Red Cross Kyoto Daiichi Hosp.*

The Rho-family GTPases play various functions, including cytoskeletal regulation and cell polarity establishment. Using *Cdc42*-KO mice under the control of the *Atoh1* promoter (*Atoh1-Cre;Cdc42*$^{flox/flox}$), we previously reported that Cdc42 plays essential roles in the maintenance of cochlear hair cells (HCs) after HC specification. Although Rac1 and Rac3 have been reported to play important roles during embryonic development of the inner ear, little is known regarding their function in cochlear HCs after specification. Here, we revealed the localization and activation of Racs at stereocilia and apical junctional complexes in primary cochlear HCs using gene gun-mediated transfection of GFP-tagged Rac plasmids and transgenic mice expressing a Rac1- fluorescence resonance energy transfer (FRET) biosensor. To examine the roles of Racs after cochlear HC specification at individual levels, we generated conditional Rac-KO mice: *Atoh1-Cre;Rac1*$^{flox/flox}$ (*Rac1*-KO) and *Atoh1-Cre;Rac1*$^{flox/flox}$*; Rac3*$^{-/-}$ (*Rac1/Rac3*-DKO) mice. Unexpectedly, both *Rac1*-KO and *Rac1/Rac3*-DKO mice exhibited normal cochlear HC morphology and normal hearing function. No hearing vulnerability was observed in young adult *Rac1/Rac3*-DKO mice even after intense noise exposure. Taken together, although Rac1 and Rac3 contribute to the early development of sensory epithelia in cochleae, they are dispensable after cochlear HC specification.

2-B-O07-3 一般演題（口頭）

# GABAergic elimination in the ventral hippocampus induces anhedonia.

Takashi Iwai, Satomi Yoshikawa, Ikumi Takagi, Ayaka Kobayashi, Misa Oyama, Shun Watanabe Mitsuo Tanabe

*Lab. Pharmacol. Sch. Pharm., Kitasato Univ.*

It has been suggested that the development of depression involves a reduction in GABAergic neurons. However, the underlying mechanism remains to be fully elucidated. In this study, we examined whether GABAergic elimination in the ventral hippocampus induces anhedonia, a core symptom of depression. Male ddY mice were bilaterally injected with saporin-conjugated anti-GABA transporter-1 antibodies (GAT1sp) in the ventral hippocampus, and then were subjected to a sucrose preference test 10 days after GAT1sp injection. Another cohort of mice received a combined chronic mild stress with ACTH treatment (ACMS) for 4 weeks, and was subjected to a sucrose preference test. Immunofluorescence was performed to detect the parvalbumin, GFAP, and pERK. In both the GAT1sp and the ACMS groups, compared to the PBS and non-stress groups respectively, there were significant decreases in sucrose preference as well as the parvalbumin-positive cell densities in the hippocampus. GFAP-positive cell densities were decreased in the ventral hippocampus of the ACMS group, but not the GAT1sp group. pERK-positive cell densities were increased in the nucleus accumbens of both the GAT1sp and ACMS groups. Together, similar to chronic stress, GABAergic elimination in the ventral hippocampus induces anhedonia accompanied by activation of ERK signal transduction in the nucleus accumbens.

**2-B-O07-4** 一般演題（口頭）

# Mice lacking GPR143, the receptor for L-DOPA, manifest enhanced sucrose preference, impaired prepulse inhibition, greater aggression and exacerbated depressive-like behavior

Yoshio Goshima, Hiromi Okatsu, Daiki Masukawa

*Dept Mol Pharmacol & Neurobiology, Yokohama City Univ*

Not Published

**2-B-O07-5** 一般演題（口頭）

# An astrocyte-derived excitatory molecule in neurological disorders

Eiji Shigetomi[1,2], Hideaki Suzuki[1,2], Yukiho Hirayama[1], Fumikazu Sano[1,2,3], Kohei Yoshihara[4], Keisuke Koga[4,5], Toru Tateoka[6], Hideyuki Yoshioka[6], Youichi Shinozaki[1,2], Hiroyuki Kinouchi[6], Kenji Tanaka[7], Haruhiko Bito[8], Makoto Tsuda[4,9], Schuichi Koizumi[1,2]

*[1]Dept Neuropharmacol, Interdiscipl Grad Sch Med, Univ Yamanashi, [2]Yamanashi GLIA center, Interdiscipl Grad Sch Med, Univ Yamanashi, [3]Dept Pediatr, Interdiscipl Grad Sch Med, Univ Yamanashi, [4]Dept Mol and System Pharmacol, Grad Sch Pharm Sci, Kyushu Univ, [5]Dept Mol and System Pharmacol, Grad Sch Pharm Sci, Kyushu Univ, [6]Dept Neurosurg, Interdiscipl Grad Sch Med, Univ Yamanashi, [7]Div Brain Sci, Inst Adv Med Res, Keio Univ Sch Med, [8]Dept Neurochem, Grad Sch Med, Univ Tokyo, [9]Dept Life Innov, Grad Sch Pharm Sci, Kyushu Univ*

Reactive astrocytes contribute to neurological disorders; however, the functional phenotypes of reactive astrocytes are poorly identified. Reactive astrocytes exhibit dysregulated $Ca^{2+}$ dynamics, which can have detrimental effects on synapses and neurons in various neurological disorders. Several molecules involved in $Ca^{2+}$ dysregulation have been identified in astrocytes. Our focus has been on the P2Y1 receptor (P2Y1R), which is activated by extracellular ATP or ADP and is implicated in neurological disorders like epilepsy, stroke, and Alzheimer's disease. To investigate the pathophysiological significance of P2Y1R upregulation in astrocytes and its downstream molecules, we utilized transgenic mice with astrocyte-specific overexpression of P2Y1R using the Tet-Off system (P2Y1OE). We focused mainly on the hippocampus and performed behavioral analysis, EEG, electrophysiology, two-photon $Ca^{2+}$ imaging, RNA-seq, and immunohistochemistry. Our findings revealed that 1) P2Y1OE leads to neuronal hyperexcitability by enhancement of astrocyte-neuron interaction, 2) P2Y1OE increases IGFBP2 expression, which enhances neuronal excitation, and 3) P2Y1R and IGFBP2 co-upregulate in disease models, such as epilepsy and stroke. Overall, P2Y1R-IGFBP2 signaling may contribute to neuronal hyperexcitability in several neurological disorders.

**2-B-O08-1** 一般演題（口頭）

# A novel molecular mechanism of apoptosis resistance regulated by S1P-aPKC signaling

Taketoshi Kajimoto[1,2], Alisha D. Caliman[2], Irene S. Tobias[2], Taro Okada[1], Caila A. Pilo[2], An-Angela Van[2], J. Andrew McCammon[2], Shun-ichi Nakamura[1], Alexandra C. Newton[2]

*[1]Dept. Biochem. Mol. Biol., Kobe Univ., [2]Dept. Pharmacol., UC San Diego*

It is well known that cancer cells have an ability of evasion of apoptosis by cellular stress like nutrient starvation. And the balance between apoptosis signal and apoptosis-resistant signal will determine the fate of cells, dead or alive. Here we found new cell signaling system that plays a role in applying brakes to cell death that in case cancer cells avoid apoptosis by cellular stress like starvation using newly developed biosensor and *in silico* docking simulation technique. The new cell signaling system is that second messenger, sphingosine 1-phosphate (S1P), directly activates a key cell signaling protein, atypical protein kinase C (aPKC).

First, we found that inhibition of aPKC induces apoptosis of cancer cells. Next, for making clear the molecular mechanism of the aPKC-induced apoptosis resistance, we generated a genetically encoded FRET reporter (aPKC-selective CKAR (aCKAR)) that allows specific visualization of aPKC activity in living cells. Using aCKAR we found that intracellular S1P induces activation of aPKC in an S1P receptor-independent manner. Biochemical studies revealed that S1P directly binds to the kinase domain of aPKC isozymes, relieving autoinhibitory constraints to activate the enzyme. *In silico* docking studies were used to identify potential binding sites for aPKC, one of which was validated by biochemical and aCKAR imaging techniques.

Now we got new insights about the player of apoptosis resistance with molecular level, and it has potential for development of new molecular-targeted agents to release brakes against cell death.

**2-B-O08-2** 一般演題（口頭）

# In vivo $Ca^{2+}$ imaging analysis of pancreatic β-cells and liver hepatocytes using transgenic mouse lines expressing ratiometric $Ca^{2+}$ sensor protein

Kazunori Kanemaru[1], Isamu Taiko[1], Kai Chen[1], Yuki Motegi[1], Yuichi Hiraoka[2,3], Toshio Miki[1], Masamitsu Iino[1]

*[1]Dept. Physiol., Nihon Univ. Sch. Med., [2]Dept. Mol. Neurosci., MRI, TMDU, [3]Lab. Genome Edit. Biomed. Res., MRI, TMDU*

Intracellular $Ca^{2+}$ signaling in pancreatic β-cells and liver hepatocytes contribute to the homeostatic regulation of living organisms by triggering the secretion of glycemia-controlling hormones and metabolic processes. $Ca^{2+}$ signals in these cells have been studied in imaging analysis using ex vivo preparations. Unlike the case of ex vivo analysis, β-cells and hepatocytes in vivo are under the influence of the autonomic nervous system, hormones, nutrients and other bioactive substances. Therefore, $Ca^{2+}$ activities in β-cells and hepatocytes under physiological conditions remain elusive. We here report in vivo $Ca^{2+}$ activities of β-cells and hepatocytes using transgenic mouse lines expressing a ratiometric $Ca^{2+}$ indicator protein, yellow cameleon-Nano50 (YC-Nano50), in β-cell or hepatocyte-specific manner. In vivo imaging analysis of these mice enabled us to visualize spatiotemporal patterns of $Ca^{2+}$ signals that were not predicted by ex vivo $Ca^{2+}$ imaging analysis. These results and our future study are expected to clarify the detailed mechanisms for homeostatic regulation by β-cells and hepatocytes.

2-B-O08-3 一般演題（口頭）

# Sex differences in podocytes and glomerular parietal epithelial cells in aging mice kidney.

Mariko Kamata[1], Kanako Hosono[1], Kou Hatanaka[1], Yoshiya Ito[1], Stuart J. Shankland[2], Hideki Amano[1]

*[1]Dept. Pharmacology. Kitasato Univ., [2]Dept. Nephrology. University of Washington*

The dialysis population has expanded rapidly with the aging of the worldwide population. Although chronic kidney disease (CKD) is more common in women than men, the male dialysis population is larger than female. In healthy aging kidney, density of podocyte and parietal epithelial cell (PEC) decreases and expressions of EMT, pericyte marker, and fibrotic thickening in PEC increase. PEC appears to be a source of new podocytes after podocyte injury. However, the relationship between sex differences and changes in PEC in aging has not been elucidated. We examined changes in glomeruli histologically using 4, 12, 18, 24, 27 months old male and female mice, assumed 26, 58, 64, 70, 79 years old in human age. The podocyte density decreased from 4 months (mo) to 12mo in both male and female mice. After 12mo, podocyte density in only female mice decreased until 24mo. The glomerular tuft area in male and female increased from 4mo to 12mo and from 4mo to 24mo, respectively. Albuminuria in male aging mice did not change, while that in female at 18mo and 27mo was higher than that at 4mo. CD44, a marker of activated PEC increased after podocyte injury and aging. In male, the CD44-positive PECs of glomeruli in outer cortical (OC) increased from 12mo and those in juxta-medullary (JM) increased from 4mo. In female, those in OC and JM increased from 24mo and 18mo, respectively. Nephrosclerosis appeared from 24om in male and 27mo in female. These results suggested that renal injury in aging occurs earlier in male mice and hyperfiltration continues older in female mice.

2-B-O08-4 一般演題（口頭）

# Suppression of autophagy contributes to enhanced necroptosis signaling in diabetic kidney after ischemia-reperfusion

Kuno Atsushi, Reo Takahashi, Yukika Saga, Yuki Tatekoshi, Ryusuke Hosoda

*Department of Pharmacology, Sapporo Medical University School of Medicine*

Background: We previously reported that impaired autophagic activation after renal ischemia-reperfusion (I/R) underlies aggravation of acute kidney injury (AKI) in type 2 diabetes (T2D). We also found that suppression of autophagy promotes necroptosis, one type of programed cell death, in the cardiomyocyte. Here, we examined whether necroptosis signaling is enhanced by suppression of autophagy in diabetic kidney after I/R.

Methods and Results: AKI was induced by unilateral nephrectomy and 30-min renal artery occlusion/24-hr reperfusion in the contralateral kidney in OLETF, T2D rats, and its control rats, LETO. In LETO, rats were pretreated with a vehicle or chloroquine (CQ; 10 mg/kg/day, 7 days), an autophagy inhibitor. In OLETF, a vehicle or rapamycin (0.25 kg/kg, IP), an activator of autophagy, was administered 30-min before reperfusion. After I/R, serum creatinine (sCr) levels were higher in OLETF than LETO. CQ pretreatment also increased sCr level in LETO. In contrast, rapamycin treatment decreased sCr levels in OLETF. Western blot analysis showed that renal levels of RIPK1, RIPK3, and MLKL, major signal proteins related to necroptosis, were increased after I/R in both LETO and OLETF. RIPK3 protein levels after I/R were higher in OLETF than LETO. In addition, RIPK3 protein levels after I/R were increased by CQ treatment in LETO. On the other hand, rapamycin treatment reduced RIPK1 and RIPK3 protein levels in OLETF.

Conclusion: The results suggest that suppression of autophagy contributes to enhanced necroptosis signals in diabetic kidney after I/R.

2-B-O08-5 一般演題（口頭）

# Mouse model of colitis increases tissue sodium and water content but decreases blood pressure

Kento Kitada, Kumar Kundo, Asadur Rahman, Akira Nishiyama

*Department of Pharmacology, Kagawa University*

The gut system absorbs water, and thereby its injury leads to body fluid loss such as diarrhea. However, the detailed effects of gut injury on body sodium/water balance and blood pressure remain to be clarified. In the present study, we examined changes in body sodium/water balance and blood pressure in a mouse model of colitis. We induced a colitis model with 3% dextran sulfate sodium (DSS) by drinking water in male C57BL6J mice. 7 days after DSS administration, colitis mice decreased body weight but significantly increased relative total body sodium and water content per tissue dry weight. Colitis mice significantly decreased urine volume per water intake coupled with increased urea content in the renal medulla. On the other hand, DSS-induced colitis mice significantly decreased blood pressure measured by a radiotelemetry system. These findings suggest that in mice with colitis, enhanced urea-driven renal water reabsorption and a decrease in urine volume induce an increase in total body tissue water content. While colitis mice increased tissue water content, blood pressure was decreased, suggesting that diarrhea caused by colitis decreases blood volume and that tissue water and blood are not equilibrated. Colitis may cause abnormal body electrolyte/water balance at the tissue level, and it may be necessary to evaluate and correct the electrolyte-water balance.

3-B-O09-1 一般演題（口頭）

# LRRK2 is involved in neutrophil chemotaxis and LRRK2 kinase inhibitor MLi -2 causes increased chemotactic activity

Yuichi Mazaki[1], Haruka Handa[2], Yoshizuki Fumoto[2]

[1]*Dept. Cell. Pharmcol., Grad. Sch. Med., Hokkaido Univ.,* [2]*Dept. Mol. Biol., Grad. Sch. Med., Hokkaido Univ.*

Neutrophils require energy supplied by mitochondria oxidative phosphorylation (OXPHOS) during chemotaxis, whereas the energy of neutrophil depends on glycolysis under normal condition. However, it is unknown the mechanism in which the energy supply changes from glycolysis to OXPHOS. Leucine-rich repeat kinase 2 (LRRK2) is partially present in the mitochondrial outer membrane fraction. *Lrrk2* deficient cells show mitochondrial fragmentation and reduction of OXPHOS activity. We previously reported that *mitofusin 2* (*Mfn2*) knockdown by shRNA suppressed mitochondrial morphological changes, OXPHOS activation and chemotactic activity upon fMLP stimulation in differentiated HL-60 (dHL-60) cells. Here, we investigated whether LRRK2, which is reported to bind to MFN2, is involved in neutrophils chemotaxis. Mouse *Lrrk2* knockout neutrophils showed reduction of chemotactic activity. *LRRK2* knockdown in dHL-60 cells showed reduction of chemotactic activity, OXPHOS activation, GTP binding activity of MFN2, and suppression of mitochondrial morphological changes upon fMLP stimulation. Furthermore, LRRK2 kinase inhibitor MLi-2 resulted in an increase in chemotactic activity. These results suggested that LRRK2 is involved in chemotaxis of neutrophils, and that LRRK2 kinase activity in particular is important for chemotaxis.

3-B-O09-2 一般演題（口頭）

# Effect of N-nonanoyl tryptamine on itch in mice with surfactant-induced eczematoid dermatitis

Tsugunobu Andoh[1], Emiri Nishida[1], Masanori Somei[2], Yasushi Kuraishi[3]

*[1]Dept. Pharmacol. Pathophysiol., College Pharm., Kinjo Gakuin Univ., [2]SOMEIYAKKO Kenkyusho, [3]URA, Wakayama Med. Univ.*

Repeated use of surfactants contained in shampoo and kitchen detergent elicits eczema with severe itch. However, there is no therapeutic agent effective for itching and skin protection. N-Nonanoyl tryptamine is an ingredient contained in cosmetics, but its function is still unknown. In this study, we investigated the effect of N-nonanoyl tryptamine (NNT) on itch in mice with surfactant-induced eczematoid dermatitis. Sodium dodecyl sulfate (SDS) was repeatedly applied once a day to the dorsal skin of mice that had been shaved in advance. NNT was repeatedly applied twice a day to the same skin region. Repeated treatment with SDS induced eczematoid dermatitis, the decrease of the water content of the stratum corneum, and scratching, which is an itch-related behavior. The treatments of NNT before SDS treatment and at least 6 h after SDS treatment on the same day inhibited significantly these symptoms elicited by SDS, compared with vehicle treatments. These results suggest that, in addition to moisturizing the skin, NNT effectives surfactant-induced eczema and itch. Therefore, it is considered that NNT is a functional cosmetic ingredient.

3-B-O09-3 一般演題（口頭）

# Suppression of itch sensation by IL-27

Daiji Sakata[1], Yusuke Nomoto[2], Masahiro Yamamoto[3], Chisa Nakashima[4], Kenji Kabashima[4], Hiroki Yoshida[5], Takuro Kanekura[2], Hiromitsu Hara[1]

*[1]Laboratory of Immunology, Department of Infection and Immunity, Graduate School of Medical and Dental Sciences, Kagoshima University, [2]Department of Dermatology, Faculty of Medicine, Kagoshima University., [3]Research Institute for Microbial Diseases, Osaka University, [4]Department of Dermatology, Graduate School of Medicine and Faculty of Medicine, Kyoto University., [5]Biomolecular Sciences, Medicine, Faculty of Medicine, Saga University*

Physiological itch is crucial for host defense because scratching behavior leads to removing potentially harmful organism from the skin. However, itch in chronic disease such as atopic dermatitis induces unpleasant effects. Recent studies have paid attention to type2 cytokines including IL-4, IL-13 and IL-31 as allergic dermatitis-associated itch mediators. Indeed, receptors of these cytokines are expressed on dorsal root ganglion neurons and the cytokines can activate and/or sensitize the neurons directly. However, immune regulation of itch sensation has not yet been fully understood.

IL-27 is an immunoregulatory cytokine which belongs to IL-12 cytokine family. IL-27 is mainly produced by antigen-presenting cells and transmits the signals via a heterodimeric receptor composed of IL-27 receptor a chain (WSX-1) and gp130. Although IL-27 suppresses Th2 and ILC2 responses, the role of IL-27 in neural system remains to be elucidated.

Here, we found that mice deficient in WSX-1 showed enhanced scratching behavior against several pruritogens. Conversely, administration of recombinant IL-27 suppressed pruritogen-induced scratching behavior in WT mice. This suppressive effect of IL-27 in itch sensation is abolished in Nav1.8-Cre WSX-1$^{flox/flox}$ mice which lack sensory neuron specific WSX-1 expression. In addition, treatment with JAK kinase inhibitor abrogates the effect of IL-27 in scratching behavior. These results imply that IL-27 acts on sensory neuron and suppresses neuronal activity by JAK kinase-dependent manner, leading to regulatory function in itch sensation.

3-B-O09-4 一般演題（口頭）

# Analgesic action of oclacitinib on postoperative pain in mice

Satoru Horie[1], Syunsuke Araki[1], Mao Kaneki[2], Manami Imamura[1], Tomoki Fukuyama[2], Yosihito Shimazu[3], Shinichiro Nakamura[1], Atsushi Tsukamoto[1]

*[1]Dept.Lab.Anim.Sci., [2]Lab.Vet. Pharmacol., [3]Lab.Food and Physiological Sci.*

Oclacitinib is a selective Janus kinase (JAK) inhibitor that targets cytokine signaling involved in pruritus and inflammation. Oclacitinib is currently used to treat canine atopic dermatitis. In addition to its anti-pruritic action, recent finding suggests that oclacitinib inhibits the response of the transient receptor potential vanilloid type 1 (TRPV1) agonist capsaicin. In this study, we assessed the effect of oclacitinib on anesthesia-induced hypothermia and postoperative inflammatory pain in mice. Male ICR mice were used in the present study. Oclacitinib (10/45 mg/kg) or vehicle was orally administered to the mice, and the core body temperature was measured under inhalant isoflurane anesthesia. Subsequently, laparotomy was performed on each mouse, and postoperative pain was assessed using the Mouse Grimace Scale (MGS) and von Frey filament test. Serum interleukin-6 (IL-6) level was measured using ELISA. Oclacitinib administration did not influence core body temperature during isoflurane anesthesia. Results of both MGS and von Frey filament test showed that oclacitinib inhibited postoperative pain in a dose-dependent manner. Mice treated with oclacitinib exhibited a decreasing trend in serum IL-6 levels compared to those treated with the vehicle. In conclusion, JAK inhibitor oclacitinib showed analgesic action in a mice-laparotomy model, suggesting its potential as an analgesic agent for the management of postoperative pain.

3-B-O09-5 一般演題（口頭）

# “Inhibitory” Cannabinoid CB2 receptors have “Excitatory” role to high-fat diet evoked systemic inflammatory response

Chihiro Nozaki[1], Haruka Hosoki[2], Andreas Zimmer[3]

*[1]Global Center for Science and Engineering, Major in Bioscience, Waseda University, [2]Department of Advanced Science and Engineering, Waseda University, [3]Institute of Molecular Psychiatry, University of Bonn*

It is widely known that cannabinoid type 2 (CB2) receptor deficiency enhances inflammatory response and further symptoms in various animal models of inflammation, allergy, or cancer. As CB2 receptors are inhibitory Gi/Go G-protein coupled receptors and as major expression site of CB2 receptors are immune cells, it is no wonder that lack of CB2 receptor might lead the exacerbated inflammation. We therefore hypothesized that lack of CB2 receptor might also enhance the high fat diet (HFD)-induced peripheral neuroinflammation. However, surprisingly, CB2 receptor knockout animals (CB2-KOs) showed the significant resistance to the HFD-induced neuroinflammation. Namely, 5-week feeding of HFD induced substantial hypersensitivity in WT mice, while tactile sensitivity of HFD-fed CB2-KO remained intact. In the same animals, we further found the robust upregulation of infiltrated macrophages, chemokine receptor CXCR4 expression and modified differentiation of splenic myeloid-derived suppressor cells (MDSCs) in HFD-fed WT animals, but not in either HFD-fed CB2 knockout mice or standard fat diet (SFD)-fed WT and CB2-KO controls. Based on these results, we will propose that CB2 receptors might have the bipolar regulatory role to chemokine receptor-mediated inflammatory response through the modulation of splenic MDSC differentiation, which in the end enhance or inhibit the development of neuroinflammation depending on its cause.

3-B-O10-1 一般演題（口頭）

# Choroid plexus epiplexus macrophage derive from parenchymal microglia

Bijay Parajuli[1,2], Eiji Shigetomi[1,2], Schuichi Koizumi[1,2]

[1]*1Department of Neuropharmacology, Interdisciplinary Graduate School of Medicine , University of Yamanashi,*
[2]*GLIA Center, University of Yamanashi*

The choroid plexus (ChP), located within each brain ventricle, are the primary source of cerebrospinal fluid (CSF), and play an important role in maintaining central nervous system (CNS) homeostasis. ChP acts as a functional immunological interface between the blood and the CSF, and functions as a gateway for the transmigration of bone marrow-derived leukocyte into brain parenchyma. Thus, ChP harbors a several immune cells like macrophages, dendritic cells, monocytes, neutrophils, and lymphocytes. Macrophages represent the largest population of immune cells in the ChP and transcriptome studies have shown that three different kind of macrophages reside in ChP of which epiplexus macrophage (ChP-epiplexus-macrophage) resemble microglia. However, the origin of ChP-epiplexus macrophage remains unknown. As fate mapping using genetic tool cannot distinguish epiplexus macrophage from microglia, we devised microglia transplantation experiment into brain parenchyma to determine the ChP-epiplexus-macrophage origin. We reveal that microglia migrate to and become ChP-epiplexus-macrophage. Our findings raise an interesting possibility that microglia may migrate from brain parenchyma to ChP to become ChP-exiplexus macrophage, where they communicate with peripheral immune cells, playing important role in regulation of physiological and pathophysological functions both in the brain and peripheral organs.

3-B-O10-2 一般演題（口頭）

# Priming effect of TF inducers on synergistic TF expression, activation of the extrinsic coagulation cascade and intra-cellular gap formation of human vascular endothelial cells

Takuma Kunieda[1], Yuhki Yanase[1], Daiki Matsubara[2], Shunsuke Takahagi[2], Koichiro Ozawa[1], Michihiro Hide[2,3]

*[1]Hiroshima Univ., [2]Hiroshima Univ., [3]Hiroshima Citizens Hosp.*

Chronic spontaneous urticaria (CSU) is known as daily recurring wheal and flare with itch for more than 6 weeks. The extrinsic coagulation system has been shown to be activated in correlation with CSU severity. We have reported that tissue factor (TF), a trigger of the extrinsic coagulation cascade, is synergistically expressed on vascular endothelial cells in response to simultaneous stimulation with TF-inducers, such as LPS and histamine. However, vascular endothelial cells are not likely to be stimulated simultaneously with TF inducers in physiological conditions. Therefore, we here investigated the priming effects of each TF inducer for synergistic TF expression in vascular endothelial cells (HUVECs). We stimulated HUVECs with a TF-inducer (first stimulation) and then stimulated cells with another TF-inducer at indicated time points (second stimulation), and detected TF expression and activity. The TF expression induced by simultaneous stimulation diminished in few hours. However, both synergistic increase of TF expression and activation of coagulation cascade were observed even when the second stimulation was added 18 hours after the first stimulation. Thus, the priming effect of TF-inducer for synergistic TF expression and activation may persist for a day or longer.

3-B-O10-3 一般演題（口頭）

# Modulation of STING signaling by advanced glycation end products depend on types and concentration of carbonyl compound

Takashi Nishinaka[1], Omer Faruk Hatipoglu[1], Hidenori Wake[1], Masahiro Watanabe[2], Takao Toyomura[2], Shuji Mori[2], Masahiro Nishibori[3], Hideo Takahashi[1]

*[1]Dept. Pharmacol., Facul. Med., Kindai Univ., [2]Dept. Pharmcol., Sch. Pharmacy, Shujitsu Univ., [3]Dept. Transl. Res. Drug Dev., Facul. Med. Dent. Pharm. Sci., Okayama Univ.*

A prolonged exposure of reducing sugar to protein leads to a non-enzymatic reaction called the Maillard reaction and results in the production of advanced glycation end products (AGEs). AGEs consist of heterogeneous compounds, and AGEs are produced by the reaction of proteins with various types of reacting carbonyl compound. We have previously demonstrated that a glycolaldehyde-derived AGE (AGE3) suppresses stimulator of interferon gene (STING)/TANK-binding kinase 1 (TBK1)/interferon regulatory transcription factor 3 (IRF3), which is a component of the innate immune system. In the present study, we investigated the effects of AGEs prepared by several carbonyl compounds on STING/TBK1/IRF3 signaling. AGEs used in the present study were numbered based on the carbonyl compound type: AGE1, derived from glucose; AGE2, derived from glyceraldehyde; AGE3, derived from glycolaldehyde; AGE4, derived from methylglyoxal; and AGE5, derived from glyoxal.

AGEs derived from aldehyde (AGE2 and AGE3) and dicarbonyl compounds (AGE4 and AGE5) suppressed cyclic GMP-AMP (cGAMP)-induced activation of STING/TBK1/IRF3 signaling, with different suppression efficiencies observed. Among the AGEs used, only AGE1 enhanced cGAMP-induced activation of STING/TBK1/IRF3 signaling. Enhancing the modulation of STING/TBK1/IRF3 signaling by AGE1 was mediated by toll-like receptor 4.

These results indicated that modulation of STING/TBK1/IRF3 signaling by prepared AGEs is dependent on the type and concentration of the carbonyl compound present.

3-B-O10-4 一般演題（口頭）

# 活性炭ケミカルフィルタを用いた清浄空気環境の曝露がマウスモデルにおけるアトピー性皮膚炎の治療効果に与える影響

Kengo Tomita[1], Chiharu Ohira[2], Himeno Saito[2], Tomoe Kurita[2], Masaki Nagane[2], Tomoki Fukuyama[2]

*[1]Inst. Tech., Shimizu Corp., [2]Lab. of Pharmacol., Sch. of Vet. Med., Azabu Univ., [3]Lab. of Biochem., Sch. of Vet. Med., Azabu Univ.*

Allergies, including atopic dermatitis (AD), are multifactor diseases, and the pathogenic mechanism is not fully understood yet. It is possible that indoor environmental changes, particularly alterations in our living conditions, have led to sympton primarily caused by Volatile Organic Compounds (VOCs). Previously, due to high levels of VOCs emitting from building materials, newly constructed residences were notably associated with the onset of 'sick house syndrome'. Intriguingly, these compounds continue to emit gases even after the disappearance of their characteristic odors. In our previous investigations identified that a Chemical Filter (CF) can effectively mitigate about 90% of VOCs, which subsequently led to a significant suppression in trans epidermal water loss and skin thickness during AD's early phases in murine models. Although the VOC removal indicated potential benefits in thwarting the onset of allergies, its synergy with pharmacological interventions remained uncharted. This study delves into the efficacies of Janus kinase (JAK) inhibitors, a drug aimed at alleviating AD-associated itching. Our results suggest a notable reduction in itch frequency and auricular lymph node cell counts within the CF group, suggesting potential symptom alleviation. This study suggests that reducing VOCs could play a crucial role in AD prevention and treatment strategies.

3-B-O10-5 一般演題（口頭）

# Prolonged oxazolone-induced pruritic dermatitis in mice with atopic dry skin

Masanori Fujii[1], Sota Oiso[1], Takeshi Nabe[2], Satoshi Tanaka[1]

*[1]Lab. Pharmacol., Div. Pharmaceut. Sci., Kyoto Pharmaceut. Univ.,[2]Lab. Immunopharmacol., Fac. Pharmaceut. Sci., Setsunan Univ.*

Atopic dermatitis is a common, chronic skin disease. In patients with atopic dermatitis, dry skin with impaired barrier function is observed even in non-lesional areas, which is called atopic dry skin. We previously showed that atopic dry skin develops when hairless mice are fed a special diet deficient in both polyunsaturated fatty acids and starch. In this study, to clarify the impact of atopic dry skin on the development of dermatitis, we compared the degree of oxazolone-induced dermatitis between normal mice and mice with atopic dry skin. The mice were sensitized by applying 5% oxazolone solution to the abdomen for two consecutive days. After one week, 0.1% or 0.5% oxazolone solution was applied to the upper dorsal skin every other day to elicit dermatitis. Oxazolone-treated normal mice showed severe dermatitis, reduced skin hydration, impaired barrier function, and itch-related scratching behavior on days 4-14 after the challenge; however, the dermatitis symptoms spontaneously resolved on day 32, even after further repeated application of oxazolone. In contrast, in mice with atopic dry skin, oxazolone-induced dermatitis was similar to that in normal mice on days 4-14, but it was prolonged on day 32. Therefore, our results indicate that atopic dry skin is an important factor that can lead to prolonged dermatitis.

3-B-O11-1 一般演題（口頭）

# Integrin-inactivating peptide FNIII14 suppresses MUC5AC secretion in airway goblet cells.

Kawakita Shosuke, Kazuhito Murakami, Fumio Fukai, Yoichiro Isohama

*Lab. of appl. pharmacol., Fac. of Pham. of Sci., Tokyo Univ. of Sci.*

Airway mucus hypersecretion is a hallmark of respiratory diseases. Recently, several reports showed that the extracellular microenvironment regulates mucus hypersecretion; however, its underlining mechanism is not well understood. Therefore, we examined the effect of FNIII14, an integrin inactivating peptide, on the production and secretion of MUC5AC, a major component of airway mucin. In this study, NCI-H292 cells, goblet-like mucus producing cells, were treated with TGF-α to induce the production of MUC5AC. Co-treatment of FNIII14 with TGF-α for 12 or 24 h did not affect TGF-α-induced MUC5AC mRNA expression. However, FNIII14 markedly suppressed MUC5AC protein level in the culture supernatant, whereas intracellular pool of MUC5AC was increased. FNIII14 also decreased MUC5AC secretion induced by ionomycin, suggesting that integrins plays important role in both spontaneous and regulated secretory process of MUC5AC. Furthermore, an inhibitor of integrin downstream signaling molecule FAK (Y15), as well as FNIII14, inhibited MUC5AC secretion. These data suggested that interaction between integrin and extracellular matrix and activation of FAK are required to obtain MUC5AC secretory property in goblet cells. These data may also provide new strategy to regulate mucus hypersecretion in airway diseases.

3-B-O11-2 一般演題（口頭）

# The regulatory effects of sleep-related neuropeptide on the upper airway muscle contraction

Yoko Irukayama[1], Hisayo Jin[2], Saki Taiji[2], Tsuyoshi Nemoto[3,7], Jundal Kim[4], Tomoya Hamamura[5], Yunosuke Ogata[5], Tomoko Misawa[6], Takashi Kanbayashi[7], Takashi Nishino[2], Shiroh Isono[2], Yositoshi Kasuya[8], Koichiro Tatsumi[6]

*[1]Med. Mycol. Res. Center, Chiba Univ.,[2]Dept. of Anesthesiology Grad. Sch. of Med. Chiba Univ.,[3]Dept. of Med. Educ. Grad. Sch. of Med. Chiba Univ.,[4]Inst. of Natural. Med. Toyama Univ.,[5]Dept. of Med. Sch. of Med. Chiba Univ.,[6]Dept. of Respirology. Grad. Sch. of Med. Chiba Univ.,[7]Univ. of Tsukuba, IIIS,[8]Dept. of Biomed. Sci.Grad. Sch. of Med. Chiba Univ.*

Although several neurotransmitters have been implicated in controlling upper airway muscles, the key neural groups contributing to these functions remain unclear. During severe hypoxia increased activity of upper airway muscles, including the genioglossus (GG), would be essential for self-resuscitation in association with gasping breaths. Impairment of this mechanism has been linked to poor outcomes in patients with alveolar hypoventilation syndrome (AHS). This study aims to elucidate the role of neuropeptides in mediating GG contraction.

Spontaneously breathing C57BL/6J mice, anesthetized with sevoflurane, underwent measurement of heart rate and GG activity. Intracerebroventricular administration of orexin or peripherally administered orexin type 2 selective receptor agonist resulted in increased GG muscle contraction. To identify the brain nucleus responsible for gasping, we analyzed activated neurons immunohistochemically using an anti-Fos antibody. Mice exhibiting gasping (2 MAC sevoflurane) showed a significantly decreased number of choline acetyltransferase/Fos double-positive cells in the pedunculopontine tegmental nucleus and laterodorsal tegmental nucleus compared to control mice {0.7 Minimum Alveolar Concentration (MAC) sevoflurane}, suggesting inhibition of cholinergic-mediated depression of GG muscle activity. Additionally, we conducted a comprehensive analysis of differentially expressed genes involved in the development and maintenance of gasping breaths, which may offer new therapeutic targets for AHS.

3-B-O11-3 一般演題（口頭）

# Search for novel therapeutic interventions against lung fibrosis using patient-derived lung myofibroblasts

Motoya Hiraki[1,2], Hidemi Ogawa[1,3], Tomoko Misawa[3], Hiroyuki Nakamura[2], Koichiro Tatsumi[3], Takuji Suzuki[3], Jundal Kim[4], Yoshitoshi Kasuya[1]

*[1]Dept. Biomed. Sci., Grad. Sch. Med., Chiba Univ., [2]Lab. Chem. Pharmacol., Grad. Sch. Pharmaceut. Sci., Chiba Univ., [3]Dept. Respirology, Grad. Sch. Med., Chiba Univ., [4]Dept. Res. and Development, Div. CBR, Inst. Natl. Med., Toyama Univ.*

Idiopathic pulmonary fibrosis (IPF) is a fatal lung disease of unknown etiology. Under pathological conditions in lungs with IPF, myofibroblasts (MyoF) play a crucial role in fibrogenesis through the accumulation of an excessive amount of extracellular matrix. To develop effective therapeutic interventions against IPF, dedifferentiation of MyoF has recently attracted considerable attention from the point of view of tissue regeneration. Here, we screened various small-molecule inhibitors using dedifferentiation assay of human lung MyoF and chose several different types of inhibitors. In addition, differentially expressed genes (DEGs) in MyoF treated with the dedifferentiating agent were comprehensively investigated by an RNA-seq/transcriptome analysis. Then, we focused on the downregulated DEGs as key candidate genes to maintain the characteristics of MyoF. To specify the therapeutic target of IPF, furthermore, the drug-target molecules as well as the downregulated DEGs were subjected to a pathway analysis. We will discuss new candidate molecules having therapeutic potential against IPF.

3-B-O11-4 一般演題（口頭）

# Ferroptosis exacerbates airway inflammation in a mouse model of papain-induced asthma

Genki Kimura, Ai Tagami, Rina Fukui, Masaki Yaita, Tomohiro Miyasaka

*Lab. Physiol. & Anat., Nihon Univ. Sch. Pharmacy*

Severe asthma is difficult to treat because it involves neutrophilic inflammation that is unresponsive to corticosteroids rather than eosinophilic inflammation. Recently, it has become clear that ferroptosis, an iron-dependent cell death, is involved in various diseases. In respiratory diseases, it is becoming clear that smoking induces ferroptosis and is involved in COPD and asthma severity. In this study, we investigated the involvement of ferroptosis in the exacerbation of airway inflammation in a mouse model of papain-induced asthma. Iron deposition in lung tissue was not observed in the papain alone-exposed group but was significantly increased in the co-exposed to papain and tobacco smoke (TS) or erastin, ferroptosis inducer, groups. The number of neutrophils and eosinophils in mice in BALF co-exposed to papain and TS/erastin was significantly increased compared to that in the papain alone-exposed group, especially the number of neutrophils was significantly increased in the TS group. These profiles provide new insights into the exacerbation of airway inflammation in severe asthma.

3-B-O11-5 一般演題（口頭）

# Involvement of VEGFR1 signaling in LPS-induced lung injury in mice

Osada Mayuko[1], Atsushi Yamashita[1], Mina Tanabe[1], Seishiroh Akinaga[1], Mariko Kamata[1], Kanako Hosono[1], Koh Hatanaka[1], Yoshiya Ito[1], Masabumi Shibuya[2], Masataka Majima[3], Hideki Amano[1]

*[1]Kitasato Univ. Pharmacol., [2]Inst. Physiol. & Med. Jobu Univ., [3]Kanagawa Inst. of Technology*

Acute respiratory distress syndrome (ARDS) is characterized by lung edema induced by increases in pulmonary vascular permeability. VEGF regulates vascular permeability; however, little is known about the role of VEGF receptor 1 (VEGFR1), a receptor for VEGF, in ARDS. Here, we examined the involvement of VEGFR1 signaling in the pathology of ARDS. ARDS was created by intra-tracheal injection of LPS to male VEGFR1 tyrosine kinase knock-out mice (TKKO) and wild-type mice (WT). Compared with WT, TKKO displayed increased lung injury histological scores and BALF levels of total protein, inflammatory cytokines (TNF, IL-1, and IL-6), and chemokine (CXCL2). VEGFR1 was expressed in the macrophages in the lung. Flow cytometry analysis of both genotypes demonstrated that alveolar macrophages were decreasing with time after LPS injection, and instead, recruited macrophages and neutrophils with higher extension were increased. Neutrophils in TKKO were greater than WT. The deletion of macrophages with clodronate liposomes (CL) in both genotypes attenuated lung injury scores and total protein, which was associated with reduced alveolar and recruited macrophages and neutrophils. But the magnitude of reduced levels of injury scores, total protein, cytokines, chemokines, and neutrophil accumulation was lower in CL-treated TKKO than that in CL-treated WT. These results suggested that attenuated acute lung injury through VEGFR1 signaling might be caused by inhibiting accumulation of neutrophils induced by cytokines and chemokines produced from macrophages.

3-B-O12-1 一般演題（口頭）

# Triterpenoid saponin from *Panax ginseng* inhibits the toxin production and increases the sensitivity to antibiotics in methicillin-resistant *Staphylococcus aureus*

Mayuko Oka[1], Sakura Tsutamoto[1], Krisna Nugraha Dendi[2], Keiichi Samukawa[3], Hiroshi Iwao[1], Yasuhiko Horiguchi[2]

[1]*Kyoto Prefectural Univ.,* [2]*RIMD, Osaka Univ.,* [3]*Osaka Metropolitan Univ.*

Red ginseng, made from the roots of *Panax ginseng* C. A. Meyer is used as a traditional medicine in East Asia to treat various diseases for more than 2000 years. Previously, we reported that red ginseng extract (RGE) containing more than90% ginsenosides might be a potential therapeutic agent for skin inflammation associated with atopic dermatitis (AD). *Staphylococcus aureus* in human skin microbiome has recently been known to be isolated with high frequency from patients with atopic dermatitis (AD). Thus, we examined whether RGE inhibits the production of virulence factors as an antibacterial activity against *S. aureus*, with the aim of applying it to the treatment of AD. RGE inhibited the expression of a/β/γ-hemolysin genes and the secretion of hemolysin toxin from *S. aureus* in dose-dependent manner. Next, since methicillin-resistant *S. aureus* (MRSA) is now commonly isolated from individuals with community-acquired infections, we examined how RGE affects antibiotic activity against MRSA. A minimum inhibitory concentration (MIC) assay revealed that RGE reduced the MIC values of b-lactam antibiotics and aminoglycoside antibiotics against two laboratory strains of MRSA by 0.03–0.25-fold. However, RGE did not alter the MIC values of fosfomycin, tetracycline, and erythromycin, suggesting that RGE acts selectively. RGE increases the bactericidal effect of antibiotics via a mechanism different from that used by triton X-100, which has been known to increases the antibacterial activity of b-lactam antibiotics. Therefore, RGE has the potential to be used as a very new antimicrobial agent for MRSA and/or *S. aureus* infection.

3-B-O12-2 一般演題（口頭）

# Goreisan modulates forskolin-induced aquaporin 2 localization change via the calcium-sensing receptor.

Keisuke Ogura, Naoki Fujitsuka, Youhei Tokita

*Tsumura Kampo Research Laboratories*

Goreisan (GRS) is a Kampo medicine that is widely prescribed for edema, migraine and diarrhea. Previous studies reported GRS to have diuretic effect and to mediate body's water balance. Aquaporin 2 (AQP2) is a water-selective channel that plays a crucial role in regulating water reabsorption in the kidneys, influencing the body's water content regulation. AQP2 on the apical membrane of the collecting duct facilitates water reabsorption via the cAMP–PKA signaling pathway. Thus, this study aimed to investigate the effects of GRS on AQP2 localization in a forskolin (FSK)-stimulated mouse inner medullary collecting duct (mIMCD-3) cell.

This study revealed that GRS dose-dependently inhibited FSK-induced cAMP production and increased intracellular $Ca^{2+}$ concentration. A selective calcium-sensing receptor (CaSR: Gi, Gq protein-coupled GPCR) inhibitor, NPS-2143, partially counteracted the effect of GRS on cAMP production and intracellular $Ca^{2+}$ concentration, indicating that GRS acts as an agonist for the CaSR. To evaluate AQP2 translocation, spheroids that recapitulate the luminal structure of the collecting ducts were established using a 3D cell culture. The colocalization area between the lumen ($ZO\text{-}1^{+}$ area) and phosphorylated AQP2 (Ser 269), which is phosphorylated with translocation, was calculated to assess AQP2 trafficking. Pretreatment with GRS partially inhibited FSK-stimulated AQP2's trafficking into the lumen. These findings suggest that the diuretic effect of GRS is attributed to its ability to modulate AQP2 localization via the CaSR.

3-B-O12-3 一般演題（口頭）

# Analysis of effect on Doxorubicin-induced cardiotoxicity by Kampo medicine

Funamoto Masafumi[1], Amiho Muramatsu[2], Miyako Ueno[2], Masaki Imanishi[2], Koichiro Tsuchiya[2], Yasumasa Ikeda[1]

*[1]Dept. of Pharmaco., Grad. Sch. of Biomed. Sci., Tokushima Univ.,[2]Dept. of Med. Pharmaco., Grad. Sch. of Biomed. Sci., Tokushima Univ.*

[Background] Doxorubicin (DOX) has serious side effects, such as cardiotoxicity. DOX tends to accumulate in the mitochondria of cardiomyocytes and cause cardiotoxicity due to mitochondrial dysfunction. cGAS, a cytoplasmic DNA sensor, synthesizes mitochondria and other self-derived free DNA fragments and induces inflammation and apoptosis via the cGAS/STING/IRF3 pathway. In this study, we identified kampo medicines that inhibit DOX cardiotoxicity via the cGAS/STING/IRF3 pathway.

[Methods & Results] To identify Kampo medicines that inhibit DOX cardiotoxicity, in vitro screening was performed using rat cardiomyoblast H9c2 cells. Approximately 40 kampo medicines were investigated for their inhibitory effect on DOX-induced apoptosis after pretreatment with 100 μg/ml each, and orengedokuto most effectivly inhibit DOX cell death was. Western blotting showed that DOX increased the levels of cleaved caspase-3, IRF3, and STING, whereas orengedokuto suppressed these levels. The cGAS inhibitor RU.521 also inhibited DOX-induced increases in cleaved caspase-3, IRF3, and STING phosphorylation levels. Next, in vivo studies were conducted using a mouse model of DOX cardiotoxicity, in which C57BL/6J mice received a single dose of 20 mg/kg DOX and a daily oral dose of 1 g/kg/day orengedokuto for five consecutive days. DOX induced IL-1$\beta$ and CXCL10 expression downstream of the cGAS/STING/IRF3 pathway, and the increased expression of cleaved caspase-3 was suppressed by orengedokuto.

[Conclusion] This study indicates that orengedokuto reduces DOX cardiotoxicity via the cGAS/STING/IRF3 pathway.

3-B-O12-4 一般演題（口頭）

# Endothelium-independent vasorelaxant effects of sudachitin and demethoxysudachitin, polymethoxyflavone from the peel of *Citrus sudachi* on isolated rat aorta

Kazuo Noguchi, Chinami Ueda, Mako Watanabe, Misaki Goma, Saki Umeda, Sawako Tabira, Koto Furuyama, Mirai Taniguchi, Aino Nagai, Haruna Kanae

*Lab. of Fac. Food Sci., Dep. of Health & Bio-Pharmaceut. Sci., Sch. of Pharm. & Pharmaceut. Sci., Mukogawa Women's Univ.*

Although polymethoxyflavones (PMFs) have been reported to exhibit various pharmacological actions, the effects of PMFs sudachitin (SDC) and demethoxysudachitin (DMSDC) from the peel of *Citrus sudachi* on the cardiovascular system have not been clarified. This study investigated the mechanisms of vasorelaxation induced by SDC and DMSDC in rat aorta. Both compounds inhibited phenylephrine-induced contractions in a concentration-dependent manner. This was also observed in the case of KCl-induced contractions although the inhibitory effect was weak. In both contractions, no differences were found in the inhibitory effects of SDC and DMSDC between endothelium-intact and -denuded aorta. The relaxant effects of SDC in endothelium-intact aortas were not affected by L-NAME or indomethacin. In endothelium-denuded aorta, propranolol did not affect the relaxant effect of SDC. Preincubation of SDC, both forskolin- and sodium nitroprusside-induced relaxation potentiated. Furthermore, the relaxant effect of SDC was not affected by the adenylate and guanylate cyclase inhibitors (SQ22536 and ODQ). phosphodiesterase (PDE) inhibitors alone, SDC alone, and a combination of PDE inhibitors with SDC exhibited relaxant effects, while the lack of any interaction between each PDE inhibitor and SDC indicated an additive effect between the two substance categories. These results suggest that SDC and DMSDC cause endothelial-independent relaxation, and that the mechanism of vasorelaxation by SDC is associated with the enhancement of cAMP- and cGMP-dependent pathways.

3-B-O12-5 一般演題（口頭）

# Goreisan prevents renal fibrosis in a mouse model of folic acid-induced chronic kidney disease

Yasumasa Ikeda[1], Aoi Suenega[1,3], Yasuyuki Seto[1,3], Masafumi Funamoto[1], Masaki Imanishi[2], Koichiro Tsuchiya[2]

*[1]Dept. Pharmacol. Tokushima Univ. Grad. Sch. Med.,[2]Dept. Med. Pharmacol. Tokushima Univ. Grad. Sch. Med.,*
*[3]Student Lab. Tokushima Univ. Fac.Med*

Background and purpose: Goreisan, a Kampo medicine, has been used to treat edema, such as heart failure, due to its diuretic effects. However, few studies have investigated the effects of Kampo medicine on chronic kidney disease (CKD). We examined the effects of Goreisan on CKD.

Methods: We examined a mouse model of folic acid (FA)-induced CKD to investigate the preventive effects of goreisan. Mice were treated with food containing Goreisan (1.5% or 3 %) at 48 h after FA intraperitoneal injection (AKI phase). Tissue samples were collected on day 14 and analyzed.

Results: Histological analysis, as well as renal function and renal injury markers, deteriorated in mice with FA-induced CKD, which was ameliorated by goreisan treatment. The increased levels of inflammatory cytokines and macrophage infiltration were also alleviated in mice treated with Goreisan. Renal fibrosis was induced by FA administration and inhibited by Goreisan treatment.

Conclusion: Goreisan may have a novel preventive effect against inflammation, oxidative stress, and fibrosis, contributing to innovation in the treatment of CKD.

3-B-O13-1 一般演題（口頭）

# Oral administration of linoleic acid immediately before glucose load slowed the elevation of postprandial glucose levels via GPR120 pathway

Yuta Yamamoto[1], Katsuya Narumi[2], Naoko Yamagishi[1], Toshio Nishi[1], Takao Ito[1], Ken Iseki[2], Masaki Kobayashi[2], Yoshimitsu Kanai[1]

*[1]Dept. Anat. Cell Biol. Wakayama Med. Univ.,[2]Lab. clinical pharmaceut. & therap., Div. Pharmasciences, Fac. Pharmaceut. Sci., Hokkaido Univ.*

Long-chain fatty acids are ligands of G-protein-coupled receptor (GPR) 40 and GPR120 as well as a major nutrient in dietary fat. Pretreatment with GPR40 agonists enhanced the secretion of insulin in response to elevating blood glucose levels, but pretreatment with GPR120 agonist did not ameliorate postprandial hyperglycemia. This study examined whether oral administration of linoleic acid (LA), a GPR40 and GPR120 agonist, immediately before glucose load would affect the elevation of postprandial blood glucose levels in rats.

The elevation of postprandial blood glucose levels was slowed by LA but not by trilinolein in rats without promotion of insulin secretion, and the effect was also observed in rats with type 1 diabetes. However, LA did not inhibit the sodium-dependent glucose transporter 1 transport in CACO-2 cells. LA slowed the gastric emptying 15 min after glucose load, and glucagon-like peptide 1 (GLP-1), but not cholecystokinin, level was elevated by LA 15 min after glucose load. GPR120 agonist ameliorated postprandial hyperglycemia but GPR40 agonist did not. Pretreatment with a GPR120 antagonist, partially canceled the improvement of postprandial hyperglycemia induced by LA.

Oral administration of LA immediately after glucose load ameliorated postprandial hyperglycemia due to slowing of gastric emptying via promotion of GLP-1 secretion. The mechanisms may be associated with GPR120 pathway.

3-B-O13-2 一般演題（口頭）

# Hepatocyte-derived EVs Might Contribute to the Inflammation of Macrophages in the Liver, and Adipose Tissue.

Yoshinori Ichihara[1,2], Yohei Sanada[2], Takahisa Nakamura[2], Takeshi Imamura[1]

*[1]Div. Pharmacol., Faculty of Med., Tottori Univ.,[2]Div. of Endocrinology, Cincinnati Children's Hospital Medical Center*

The enhancement of chronic inflammation via interorgan communication like liver-adipose tissue is one of the pathological bases for the development of obesity. Although small extracellular vesicles (sEVs) are suggested to play a critical role in intercellular communications and glucose intolerance in obese and diabetic conditions, the exact target tissue/cells for hepatocyte-derived sEVs (HsEVs) *in vivo* and their contribution to chronic inflammation are not clear. Therefore, we have generated a new mouse model to track HsEVs by scarlet, red fluorescent protein, *in vivo*.

We observed high numbers of scarlet-positive (Sc+) CD45-positive cells without expressing scarlet mRNA in the pancreas, and visceral adipose tissue (eWAT), suggesting Sc+ HsEVs-recipient cells are contained in these tissues. The scRNA-seq determined that macrophages/monocytes are the major HsEVs-recipient cell types. High-fat diet (HFD) increased mRNA expression for sEV biogenesis in the liver and Sc+ HsEVs ratio in the serum in mice. Sc+ macrophages exhibited higher levels of TNFa compared to scarlet-negative macrophages, and this level in the Sc+ macrophage was further enhanced in the HFD condition.

These data suggest that the hepatocytes in obesity are involved in generating pro-inflammatory sEVs, which may contribute to macrophage activation in the eWAT.

3-B-O13-3 一般演題（口頭）

# Thyroid hormone insufficiency in neonatal period causes the circadian rhythm alteration as well as the Sox2 upregulation in the developing suprachiasmatic nucleus in mice

Kana Ohuchi, Kanae Satoh, Takahiro Moriya

*Dept. Pharmacol. Sch., Pharmaceut. Sci., Ohu Univ.*

Thyroid hormone plays a critical role in development of central nervous system, such as neurogenesis, synaptogenesis and myelination. However, little is known about the role of the thyroid hormone during the perinatal period in the development of the mammalian circadian clock located in the hypothalamic suprachiasmatic nucleus (SCN). In this study, we examined the effects of the pharmacological insufficiency of thyroid hormone in the perinatal period on the wheel-running circadian rhythm in the adulthood as well as the gene expression in the neonatal SCN of mice. The pregnant mice were given antithyroid agents from ED17 to 14th day after the delivery and their offspring was normally grown and treated as congenic hypothyroid (CH) mice. CH mice at 8 wk ages showed the shorter period in the circadian behavioral rhythm in the constant darkness compared with control mice. The period of Per2::luciferase reporter rhythm in the explant SCN culture was shorter in CH mice than that from control mice. The mRNA expression of Sox2 gene, which is known to be critical for the normal development of the SCN clock, was upregulated in CH mice. Furthermore, the daily administration of thyroxin all ameliorated the phenotype in CH mice. These results impact the important roles of thyroid hormone in the circadian clock development.

3-B-O13-4 一般演題（口頭）

# Possible roles of mitochondrial protein p13 in the control of white adipose tissue amount

Masafumi Noguchi[1], Satomi Hara[2], Keiko Iwata[1], Hitoshi Hashimoto[2,3,4,5], Norihito Shintani[1,2]

*[1]Sch. Pharmaceut. Sci., Wakayama Med. Univ.,[2]Grad. Ach. Pharmaceut. Sci., Osaka Univ.,[3]United Grad. Sch. Child Dev., Osaka Univ.,[4]Div. Biosci., Inst. Datability Sci., Osaka Univ.,[5]Inst. Open Transdiscip. Res. Initiatives, Osaka Univ.*

Mitochondria are involved in various cellular functions such as energy production and apoptosis, and their dysfunction has been regarded as one of the causes of metabolic disorders such as diabetes. We recently identified the mitochondrial protein p13 with a molecular weight of 13 kDa based on the decreased expression in pancreatic islets of Langerhans in type II diabetic mice, and have been conducting research using systemically deficient mice (p13-/- mice). Here, we investigated the role of p13 in white adipose tissue (WAT) pathophysiology via multiscale omics analyses in vivo and in vitro. First, we measured the weight of various tissues in the whole body and found that the weight of white adipose tissue was markedly decreased in p13-/- mice. Moreover, as a result of comprehensive histochemical analysis, selective and marked abnormalities were observed in p13-/- WAT compared to the other tissues, and especially, a marked reduction in the size of adipocytes and lipid droplets were observed. Besides, RNA-seq and qRT-PCR analyses revealed decreases in lipid synthesis genes and increases in lipid degradation genes in p13-/- WAT, suggesting the role of p13 in lipid metabolism. In contrast, there was no significant difference in adipose differentiation marker genes, and we also confirmed that p13 deficiency did not affect adipose differentiation in an adipocyte differentiation model using mouse embryonic fibroblasts (MEFs). Serum parameter analysis revealed high levels of total ketone bodies and low levels of blood glucose, lipase, triglycerides, and HDL-C. Besides, p13-/- mice had lower steady-state insulin levels and lower levels of insulin-antagonizing corticosterone. Taken together, the present results suggest that WAT is markedly decreased in p13-/- mice due to abnormal hormone homeostasis, and that endogenous p13 may play an important role in regulating hormon release and lipid metabolism.

3-B-O13-5 一般演題（口頭）

# Functional Analysis of Sphingosine Kinase 1 in Brown Adipose Tissue

Morishige Jun-ichi[1], Kazuaki Yoshioka[2], Naoto Nagata[1], Tamotsu Takana[3], Yoh Takuwa[2], Hitoshi Ando[1]

*[1]Dept. Cell. Mol. Func. Anal. Kanazawa Univ., [2]Dept. Physiol. Kanazawa Univ., [3]Grad. School of Tech., Industrial and Soc. Sci., Tokushima Univ.*

Objective: Sphingosine 1-phosphate (S1P) is implicated in brown adipose tissue (BAT) formation and energy consumption; however, the mechanistic role of sphingolipids, including S1P, in BAT remains unclear. Here, we sought to elucidate the physiological significance of sphingolipids in BAT.

Methods: Global sphingosine kinase 1 (SphK1)-deficient mice and their wild-type littermates were housed at both room temperature and cold environment to capture the physiological phenotypes. Subsequently, qRT-PCR, immunostaining, immunoblotting, and determinations of S1P and triglyceride were performed in isolated BAT and cultured brown adipocytes.

Results: In BAT, SphK1 was localized largely in the lysosomes of brown adipocytes, and SphK1 and its product S1P levels were upregulated in cold-activated BAT. Genetic deletion of *Sphk1* resulted in a reduced number of brown adipocyte lysosomes, which was accompanied by impairment of lysosomal functions, including proteolytic activity and motility. Interestingly, *Sphk1*$^{-/-}$ mice exhibited mild hypothermia and greater triglyceride content with larger lipid droplets dominating in the brown adipocytes. Triglyceride accumulation in brown adipocytes induced by the lysosome inhibitor chloroquine was blunted in *Sphk1*$^{-/-}$ mice compared to wild-type mice, suggesting a reduced lysosome-mediated lipolysis in *Sphk1*$^{-/-}$ mice.

Conclusion: Our results indicate a novel role of SphK1 in lysosomal integrity, which is required for lipolysis and thermogenesis in BAT.

3-B-O14-1 一般演題（口頭）

# Overcoming multidrug resistance in cancer by targeting Hsp70-interacting proteins

Masako Tanaka[1], Masayuki Shiota[2], Susumu Imaoka[1]

*[1]Dept. Biomed. Chem., Sch. Biol. Environ. Sci., Kwansei Gakuin Univ., [2]Dept. Mol. Biol. Med., Grad. Sch. Med., Osaka Metropolitan Univ.*

Multiple drug resistance, a phenomenon in which cancer cells that acquire resistance to one type of anticancer drug are also resistant to several other drugs, often quite different in both structure and mechanism of action, has been studied. Our previous studies have identified a novel oxaliplatin resistance factor by analyzing heat shock protein 70 (Hsp70) interacting proteins. Hsp70, a stress response molecule, has been reported to be involved in the resistance to several anticancer drugs, including oxaliplatin. This study analyzed Hsp70-interacting proteins and then identified novel molecules that contribute to multidrug resistance. To identify Hsp70 interactors critical for oxaliplatin, 5-fluorouracil, paclitaxel, and irinotecan resistance in human gastric cancer cells, OCUM-2M, we performed mass spectrometry-based proteomic analysis using affinity purification with anti-Hsp70 antibodies. This led to the identification of six Hsp70 interactors common to the four resistant cell lines. These six candidates were then subjected to RNAi screening to assess drug sensitivity. Two candidates contributed to cell proliferation, while the other four candidates increased sensitivity to anticancer drugs. These results suggest that Hsp70 interactors cause multiple drug resistance.

3-B-O14-2 一般演題（口頭）

# Carbidopa and benserazide that inhibit cystathionine-β-synthase, an $H_2S$-forming enzyme, suppress the viability of both bortezomib-sensitive and -resistant multiple myeloma cells

Fumiko Sekiguchi[1], Haruka Moriguchi[1], Yukiho Fukushima[1], Yuriko Iba[1], Maho Tsubota[1], Shiori Hiramoto[1], Takuya Okada[2], Naoki Toyooka[2], Hirokazu Tanaka[3], Takashi Ashida[3], Itaru Matsumura[3], Atsufumi Kawabata[1]

*[1]Lab. Pharmacol. Pathophysiol., Fac. Pharm., Kindai Univ., [2]Grad. Sch. Sci. Technol., Univ. Toyama,[3]Div. Hematol. Rheumatol., Kindai Univ. Fac. Med.*

Given the involvement of $H_2S$, a gaseous mediator, in multiple myeloma (MM) cell proliferation and survival, we evaluated effects of benserazide and L-carbidopa, aromatic L-amino acid decarboxylase (AADC) inhibitors capable of inhibiting cystathionine-$\beta$-synthase (CBS), an $H_2S$-forming enzyme, on the viability of human MM-derived KMS-11 and bortezomib (BTZ)-resistant KMS-11/BTZ cells. Three different $H_2S$-forming enzymes, i.e. CBS, cystathionine-$\gamma$-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3-MST), were comparably expressed in KMS-11 and KMS-11/BTZ cells. Application of BTZ at 10 nM for 24 h upregulated CBS, but not CSE or 3-MST, in KMS-11 cells. Benserazide and L-carbidopa, as well as aminooxyacetic acid, a well-known CBS inhibitor, markedly reduced viability of both MM cells, an effect mimicked by D-carbidopa that inhibited CBS, but not AADC. CSE and 3-MST inhibitors had relatively weak and little anti-MM effects, respectively. The cytotoxic effect of L-carbidopa was reversed by $Na_2S$, an $H_2S$ donor. Benserazide and L- and D-carbidopa reduced phosphorylation of NF-$\kappa$B p65 in KMS-11 cells. Our data suggest that the CBS/$H_2S$/NF-$\kappa$B pathway is involved in the survival of BTZ-sensitive and -resistant MM, and that D-carbidopa, an inhibitor of CBS, but not AADC, would be useful to treat BTZ-resistant MM.

3-B-O14-3 一般演題（口頭）

# SN38-BGLs: Novel hydrophilic camptothecin derivatives suppress tumor growth without diarrhea in murine xenograft model.

Licht Miyamoto[1,2], Yuki Tsuchihashi[2], Shinji Abe[3], Toshihiro Izumi[3], Aya Hatano[2], Masaki Imanishi[2,4], Yasumasa Ikeda[4], Koichiro Tsuchiya[2]

*[1]Pharmacol. & Food Sci., Dept. Nutr. & Life Sci., Fac. Health & Med. Sci., Kanagawa Inst. of Technology,[2]Dept. of Med. Pharmacol. Inst. of Biomed. Sci., Grad. Sch. of Tokushima Univ.,[3]Dept. of Clin. Pharm. Pract. Pedagogy, Inst. of Biomed. Sci., Grad. Sch. of Tokushima Univ.,[4]Dept. of Pharmacol. Inst. of Biomed. Sci., Grad. Sch. of Tokushima Univ.*

Camptothecin derivatives, such as CPT-11 (irinotecan), possess potent antitumor properties but are often hampered by their hydrophobic nature, leading to severe diarrhea as a common adverse effect. To address this challenge, we designed and synthesized novel highly hydrophilic camptothecin derivatives by conjugating SN38 with branched glycerol trimer (SN38-BGL). This unique strategy aimed to enhance the therapeutic benefits while minimizing the adverse effects. In murine xenograft models of human lung cancer, SN38-BGLs exhibited comparable or slightly superior tumor-suppressing efficacy compared to CPT-11 without the onset of early or late diarrhea. Additionally, histological analysis revealed that SN38-BGL treatment resulted in longer villi in the jejunum and ileum compared to CPT-11, indicating that SN38-BGL is less harmful. Digestion by liver microsome ex vivo did not yield SN38 but a few other molecules, suggesting possible involvement of other active metabolites than SN38. Our findings suggest that SN38-BGLs represent a promising class of hydrophilic camptothecin derivatives with the potential to mitigate severe diarrhea while maintaining antitumor efficacy, offering new prospects for pharmacological interventions.

3-B-O14-4 一般演題（口頭）

# Novel pharmacotherapy targeting NF-κB signal for triple-negative breast cancer patients with loss of CYLD expression

Hitomi Arakaki[1], Akari Kuwano[1], Chika Hirai[1], Chica Saigo[1], Ayumi Kanemaru[1], Mitsuhiro Hayashi[3], Hideyuki Saito[2], Yuki Narita[2], Hirofumi Jono[2]

*[1]Dept. of Clin. Pharmaceut. Sci., Grad. Sch. of Pharmaceut. Sci, Kumamoto Univ., Kumamoto, Japan., [2]Dept. of Pharm., Kumamoto Univ. Hosp., Kumamoto, Japan., [3]Dept. of Breast and Endocrine Surgery, Grad. Sch. of Med. Sci., Kumamoto Univ., Kumamoto, Japan*

【INTRODUCTION】Triple negative breast cancer (TNBC) is the most aggressive form of breast cancer. Since TNBC exhibits a significantly lower 5-year survival rate than other subtypes, development of novel therapeutic strategies for TNBC is urgently needed. Tumor suppressor gene Cylindromatosis (CYLD) is associated with acquisition of malignant traits in various malignant tumors as a negative regulator for intracellular signals, such as nuclear factor-κB (NF-κB) signaling. The aim of this study was to clarify the clinical and biological significance of reduced CYLD expression in TNBC and to explore novel pharmacotherapy CYLD-negative poor-prognosis TNBC patients.

【METHODS】We knocked-downed CYLD expression by CYLD-specific siRNA in TNBC cells (MDA-MB-231 cells), and determined the therapeutic effects of anti-tumor drugs by evaluating malignant characteristics, such as, migration ability and anticancer drug sensitivity.

【RESULTS】In CYLD knock-downed TNBC cells, cell migration and drug resistance for epirubicin and 5-FU, standard drugs for TNBC, were promoted via hyper-activation of NF-kB signaling. Indeed, NF-kB inhibitor (BAY11-7085) significantly suppressed the cell migration and cell viability caused by CYLD knock-down. Furthermore, therapeutic effects of clinically approved NF-kB inhibitors, such as, denosumab and bortezomib, were evaluated in CYLD knock-downed TNBC cells. In addition to the therapeutic effect of denosumab on cell migration, interestingly, bortezomib showed significant inhibitory effect on both cell viability and cell migration.

【DISCUSSION】Loss of CYLD expression enhanced the cell migration and drug resistance through hyper-activation of NF-kB signaling. Both clinically approved NF-kB inhibitors exhibited therapeutic effects, in particular, bortezomib might be more effective for TNBC cells with loss of CYLD expression by suppressing both cell viability and cell migration.

【CONCLUSION】Pharmacotherapy targeting NF-kB signaling may have potential to be novel therapeutic strategy for poor prognosis TNBC patients with loss of CYLD expression.

3-B-O14-5 一般演題（口頭）

# Clioquinol affects the mitochondrial respiratory chain complex IV

Masato Katsuyama

*Radioisotope Ctr., Kyoto Pref. Univ. Med.*

Clioquinol (Quinoform) was extensively administered to treat indigestion and diarrhea in the mid-1900s. However, it was withdrawn from the market in Japan because its use was epidemiologically linked to an increase in the incidence of subacute myelo-optic neuropathy (SMON). SMON is characterized by the subacute onset of sensory and motor disturbances in the lower extremities with occasional visual impairments, which are preceded by abdominal symptoms. The underlying mechanisms of clioquinol toxicity, however, have not been elucidated in detail. We previously reported that clioquinol induced oxidation of the copper chaperone ATOX1, leading to the impairment of the functional maturation of a copper-dependent enzyme dopamine-$\beta$-hydroxylase and the inhibition of noradrenaline biosynthesis. Effects of clioquinol on expression levels of other copper-related proteins were investigated. Quantitative PCR demonstrated that clioquinol decreased mRNA levels of SCO1 and SCO2, copper chaperones for mitochondrial respiratory chain complex IV (cytochrome c oxidase), and of COX18, a membrane insertase of a complex IV component. In clioquinol-treated cells, the assembly and activity of complex IV were suppressed. Thus, clioquinol was suggested to suppress ATP synthesis through this pathway, leading to neuronal cell death.

3-B-O15-1 一般演題（口頭）

# Hypoxia increases intracellular BH4 levels under inflammatory cytokine-stimulated human umbilical vein endothelial cell (HUVEC).

Kazuhisa Ikemoto, Taiki Kano, Yui Suganuma, Chiho Ichinose, Kazunao Kondo

*Department of Pharmacology, Fujita Health University School of Medicine*

Tetrahydrobiopterin (BH4) is an essential cofactor for nitric oxide synthase (NOS), thus BH4 might play a key role for maintaining endothelial functions. Here we examined whether hypoxia affects intracellular BH4 levels under inflammatory cytokine-stimulated human umbilical vein endothelial cells (HUVEC). IFN-gamma and TNF-alpha (each 15 ng/mL) markedly increased BH4 levels after incubation for 24h. Hypoxia (3%, $O_2$) significantly caused further increase of the BH4 levels than those in normoxia. Hypoxia also caused decreasing levels of BH2, which inhibits NOS activity and causes production of superoxide anion. Contrary to the increased BH4 levels, hypoxia reduced mRNA expression of GTP cyclohydrolase 1 (GCH1), the rate limiting enzyme in BH4 biosynthesis. As to the increased intracellular BH4 levels, we considered two possibilities for the reason: (1) elevated activity of dihydrofolate reductase, which is able to reduce BH2 to BH4; (2) turn back of once released BH4 into cells by autocrine or paracrine manner, since degradation of BH4 might less occur under hypoxic condition. The decrease of GCH1 mRNA expression suggested the presence of a feedback mechanism in BH4 biosynthesis other than the feedback mediated by GTP cyclohydrolase 1 feedback regulatory protein.

3-B-O15-2 一般演題（口頭）

# Inhibitory effects of corosolic acid on cell proliferation in pulmonary arterial hypertension

Aya Yamamura, Hossain Alamgir, Rie Takahashi, Motohiko Sato

*Dept. Physiol., Aichi Med. Univ.*

Corosolic acid (CRA) is a natural compound extracted from leaves of Banaba and exerts anti-inflammatory, anti-diabetic, anti-hyperlipidemic, anti-oxidant, and anti-tumor effects. We have recently demonstrated that CRA inhibits the expression of STAT3 and ameliorates the development of pulmonary arterial hypertension (PAH). In the present study, we focused on platelet-derived growth factor receptor (PDGFR) signaling, which enhances in PAH patients, and examined the downregulating mechanism of STAT3 expression by CRA. The expression level of PDGFR$\beta$ was higher in pulmonary arterial smooth muscle cells (PASMCs) from idiopathic pulmonary arterial hypertension (IPAH) patients than in normal PASMCs. Increased expression of PDGFR$\beta$ in IPAH-PASMCs was reduced by the treatment with CRA in a time-dependent manner. The expression of two downstream pathways of PDGFR$\beta$ signaling, STAT3 and NF-$\kappa$B, was also upregulated in IPAH-PASMCs, which was attenuated by CRA. In addition, the phosphorylation levels of PDGFR$\beta$ and STAT3 were reduced by CRA. The excessive proliferation and migration of IPAH-PASMCs following PDGF stimulation were inhibited by CRA. In conclusion, CRA inhibits the development of PAH via the downregulation of PDGFR$\beta$-STAT3 and PDGFR$\beta$-NF-$\kappa$B signaling pathways.

3-B-O15-3 一般演題（口頭）

# YAP-induced upregulation of GLUT1 promotes adaptive cardiac hypertrophy during acute pressure overload

Toshihide Kashihara[1], Tsutomu Nakahara[1], Junichi Sadoshima[2]

*[1]Kitasato Univ. Sch. of Pharmceut. Sci. Dept. of Mol. Pharmacol., [2]Rutgers New Jersey Med. Sch. ▪Cardiovasc. Res. Inst. ▪Dept. of Cell Bio. and Mol. Med.*

Yes-associated protein 1 (YAP), a transcriptional co-activator, is known to regulate cell growth and organ size. We have shown previously that YAP is activated in response to acute pressure overload (PO), and that YAP cardiac-specific heterozygous knockout (YAPchKO) mice are suppressed adaptive cardiac hypertrophy with inhibition of GLUT1 upregulation during acute PO. Furthermore, we found that YAP promotes glycolysis by upregulating GLUT1 in cultured rat ventricular myocytes. Glycolysis is intimately involved in cell growth. Thus, we examined whether AAV-mediated GLUT1 overexpression rescues adaptive cardiac hypertrophy during acute PO in YAPchKO mice. Wild-type (WT) or YAPchKO mice were injected with AAV-control or AAV-GLUT. After 14 days, these mice were subjected to sham or transverse artic constriction (TAC) for 7 days. TAC induced adaptive cardiac hypertrophy in WT mice injected with both AAVs. In contrast, left ventricular (LV) dysfunction, LV dilation, and inhibition of cardiac hypertrophy were observed in YAPchKO mice injected with AAV-control after TAC. AAV-GLUT1 injection successfully rescued GLUT1 upregulation, LV function, and cardiac hypertrophy in YAPchKO mice after TAC. These results suggest that YAP-induced upregulation of GLUT1 plays a critical role in promoting adaptive cardiac hypertrophy during acute PO.

3-B-O15-4 一般演題（口頭）

# Effect of CaMKII on Atrial Fibrillation in Early Stage of High-Fat Diet-induced Obesity

Tatsuya Sawano[1], Junichiro Miake[1], Takuya Tomomori[2], Akihiro Okamura[2], Priyono Agung[1], Yoshinori Ichihara[1], Keiko Nagata[1], Takeshi Imamura[1]

*[1]Division of Pharmacology, Faculty of Medicine, Tottori University,[2]Department of Cardiovascular Medicine, Endocrinology and Metabolism, Faculty of Medicine, Tottori University*

Background: The prevalence of atrial fibrillation (AF), particularly associated with obesity, is on the rise worldwide. $Ca^{2+}$/calmodulin-dependent protein kinase (CaMKII) is activated in the advanced condition of atrial remodeling. However, it is unclear whether CaMKII is activated and affects vulnerability to AF in the early phase of obesity. In this study, we examined the involvement of CaMKII in the inducibility and duration of AF in the early stage of diet-induced obese mice.

Methods: Mice were fed a normal chow diet (NCD) or high-fat diet (HFD). Following diet-loading for 2 weeks, HFD-fed mice were administrated CaMKII inhibitor. Induction of AF was performed by transesophageal atrial burst pacing. Furthermore, we evaluated the expression of CaMKII, blood pressure, and atrial fibrosis.

Results: HFD-fed mice increased the inducibility of AF compared to NCD mice. In addition, treatment with the CaMKII inhibitor in HFD-fed mice reduced the inducibility of AF. Expression of phosphorylated CaMKII is increased in HFD-fed mice. Inhibition of CaMKII didn't have effects on blood pressure, and fibrosis.

Conclusion: Inhibition of CaMKII reduced the inducibility of AF in the early phase of obesity without affecting atrial structural remodeling, suggesting that CaMKII is a factor that contributes to AF from the early stage of obesity.

3-B-O15-5 一般演題（口頭）

# The up-regulation of Neuregulin-1-ErbB signaling contributes to preventing the onset of systolic dysfunction in diabetic cardiomyopathy

Yoshinori Mikami, Fumiki Iwase, Daisuke Ohshima, Taichiro Tomida, Satomi Adachi-Akahane

*Dept. Physiol., Fac. Med., Toho Univ.*

Diabetic cardiomyopathy (DMCM) is characterized by an early left ventricular (LV) diastolic dysfunction and subsequent progression to systolic dysfunction. In this study, we hypothesized the downregulation of cardioprotective factors is involved in the pathogenesis of DMCM. We aimed to elucidate the mechanisms underlying the DMCM development. In the streptozotocin (STZ)-induced type 1 diabetic model mice 4 weeks after STZ injection (STZ -4W), diastolic function was impaired without systolic dysfunction. Counter to expectations, the serum levels of neuregulin-1 (NRG1) were significantly up-regulated in the STZ-4W group compared to the control group. NRG1 expression was increased in the ventricle, kidney, and liver in the STZ-4W group. In the ventricles, NRG1 was localized in the vascular endothelial cells, endocardium, and epicardium. To clarify the physiological role of NRG1, trastuzumab (TRZ), an antibody against NRG1 receptor ErbB2 (HER2), was administered to mice. The systolic function, T-tubule structure, and the accumulation of $Ca_V1.2$ at the junctional structure were significantly impaired in the TRZ-injected STZ-4W mice compared to STZ-4W mice. These results suggest that the compensatory up-regulation of NRG1-ErbB signaling contributes to maintaining the LV systolic function in the early stage of DMCM.

3-B-O16-1 一般演題（口頭）

# Organ Specificity of Mesenchymal Stromal Cells Underlie Homeostatic Tissue Maintenance of Multicellular Organisms.

Kurosawa Tamaki[1,7], Noriyuki Kaji[2], Momo Goto[1], Takashi Chaen[1], Taiki Mihara[1], Madoka Uezumi[3], Yuki Yoshimoto[4], Keitaro Minato[5], Eiji Hase[6], Yumiko Oishi[7], Hiroyuki Koike[7], Ichiro Manabe[8], Akiyoshi Uezumi[3], Masatoshi Hori[1]

*[1]Dept. of Vet. Pharmacol. Grad. Sch. of Agr. and Life Sci., Univ. of Tokyo,[2]Vet. Pharmacol., Sch. of Vet. Med., Azabu Univ., [3]Div. of Cell Heterogeneity, Med. Inst. of Bioregulation, Kyushu Univ.,[4]Sec. of Mol. Craniofac. Embryol. Grad. Sch. of Tokyo Med and Dent. Univ.,[5]Dept. of Regen. and Transplant Med., Div. of Orthopedic Surg., Grad. Sch. of Med. and Dent. Sci., Niigata Univ., [6]Div. of Interdiscip. Researches for Med. and Photonics, Inst. of Post-LED Ph otonics, Tokushima Univ.,[7]Dept. of Med. Biochem., Grad. Sch. of Med., Tokyo Med. and Dent. Univ., [8]Dept. of Systems Med., Grad. Sch. of Med., Chiba Univ.*

In biology, embryology has explained how multiple cell types arise from a fertilized egg and organs are formed. In the following chapter, we seek to explain the mechanisms of how organs from multiple cells are maintained in a homeostatic state. Understanding this state is essential for maintaining the health of us.

In general, organs consist of two major groups of cells: parenchymal cells and stromal cells. The properties of organs have been understood through the function of parenchymal cells. For example, hepatocytes are studied when investigating the function of the liver, and myocytes are studied as well. The interstitium, on the other hand, has been regarded as a scaffold for the parenchyma and as similar independent of organ origin. However, we focused on mesenchymal stromal cells (MSCs) for understanding organs.

In this study, we performed a comparative analysis of MSCs over six organs. We revealed the organ-specific character of MSCs and identified a muscle-maintaining functional molecule whose expression is reduced by aging. Its knockout mice exhibited a loss of muscle mass and muscle strength at a young age. The concept that MSCs maintain organ function, as demonstrated by this study, can be extended to other organs. In this presentation, we also would like to discuss the possibilities for future research development from a new perspective of 'understanding organs from the interstitium'.

3-B-O16-2 一般演題（口頭）

# Investigation of mucosal absorbing water of the rat bladder using “inside-out” samples

Naoki Aizawa, Tomoe Fujita

*Dept. Pharmacol. Toxicol., Dokkyo Medical Univ.*

Aims: It has been suggested that the urinary bladder has a physiological role for absorbing water, but there were few studies published. In contrast, the bladder mucosa plays the role of a barrier mechanism that does not allow urine to penetrate. In this study, we aimed to investigate mucosal permeability of rat urinary bladder by using newly established bladder “inside-out” preparation.

Methods: Female adult Sprague-Dawley rats were used. Using isoflurane anesthesia rats were sacrificed and whole bladder was isolated. This whole bladder was used as normal (N=12) and inside-out (N=11) samples, and inside-out sample was reversed the bladder from the bladder top. For monitoring of intravesical pressure and instillation of Krebs solution, a catheter (PE-50) was inserted through the urethra and fixed with surgical suture at the bladder neck. The bladder was fixed vertically in a 30 ml organ bath with Krebs solution gassed with 5% $CO_2$ and 95% $O_2$ at 37°C. The bladder was instilled with Krebs solution at a rate of 6 ml/h. until the intravesical pressure reached 30 $cmH_2O$. The bladder was kept under an isovolumetric condition and allowed to stabilize for 5 min (approximately around 15-20 $cmH_2O$), and then high $K^+$ (KCl: 50 mM) and acetylcholine (ACh: 10 $\mu$M) were added into the organ bath, and the intravesical pressure was recorded.

Results: In the normal bladder samples, intravesical pressure was remarkably increased with KCl (42.4 ± 3.3 $cmH_2O$) and ACh (33.3 ± 1.9 $cmH_2O$). In contrast in the inside-out bladder samples, the intravesical pressure was only slightly increased with KCl (1.0 ± 0.3 $cmH_2O$) and ACh (1.8 ± 0.2 $cmH_2O$).

Conclusions: Bladder normal samples showed remarkable increased responses of the intravesical pressure to KCl and ACh, but inside-out samples did not show such responses. The present study revealed that the bladder detrusor smooth muscle potentially has water (urine) permeability, whereas bladder mucosa has robust barrier mechanisms for preventing absorb water (urine) in at least normal rat.

3-B-O16-3 一般演題（口頭）

# Novel CACNA1S mutations for malignant hyperthermia enhance depolarization-induced $Ca^{2+}$ release

Takashi Murayama[1], Takuro Numaga-Tomita[2], Hirotsugu Miyoshi[3], Keiko Mukaida[3], Taichiro Tomida[4], Nagomi Kurebayashi[1], Takuya Kobayashi[1], Tsutomu Nakada[2], Satomi Adachi-Akahane[4], Yasuo M. Tsutsumi[3], Mitsuhiko Yamada[2], Takashi Sakurai[1]

*[1]Dept Pharmacol, Juntendo Univ, [2]Dept Mol Pharmacol, Shinshu Univ, [3]Dept Anesthesiol Crit Care, Hiroshima Univ, [4]Dept Physiol, Fac Med, Toho Univ*

Malignant hyperthermia (MH) is a life-threatening genetic disorder in which general anesthetics cause massive $Ca^{2+}$ release from the sarcoplasmic reticulum in skeletal muscle. While most MH mutations have been found in the ryanodine receptor type 1 (RyR1), other genes are also associated with MH. We have recently identified several novel mutations in CACNA1S, the $\alpha$1S subunit of the dihydropyridine receptor, from MH-susceptible patients. In this study, we performed a functional analysis of MH mutants using the reconstituted depolarization-induced $Ca^{2+}$ release (DICR) platform. HEK293 cells expressing RyR1 were infected with baculovirus containing genes essential for DICR (CACNA1S, $\beta$1a, JP2, Stac3, and Kir2.1). DICR was induced by depolarization with different concentrations of potassium ($K^+$) solutions. Cells expressing two CACNA1S mutants showed greater sensitivity to $K^+$ than WT cells. Consistently, charge movements were shifted to more hyperpolarizing potentials. These results suggest that CACNA1S MH mutations shift the DICR to a more hyperpolarizing potential. The two mutant cells showed greater sensitivity to caffeine than WT cells. Interestingly, the enhanced caffeine sensitivity was abolished by hyperpolarizing the resting membrane potential or by removing Stac3. Possible mechanisms of MH pathogenesis are discussed.

3-B-O16-4 一般演題（口頭）

# Morphological study of actin remodeling with Spingosylphosphorylcholine in smooth muscle cell

Hideyuki Tanaka

*Dept, Anatomy, Teikyo Univ, sch,Med*

We have been searching for mechanism to induce smooth muscle contraction migration that involved actin remodeling and associated with phosphorylation of Myosin light chain (MLC) of smooth muscle myosin. We report that Spingosylphosphorylcholine (SPC) stimulates ATPase activity of vascular smooth muscle (A7r5) cell and increased migration of A7r5 cells and enhanced appearance magnapodia consisting of actin membrane structure. After SPC stimulation, mitogen-activated protein kinases (MAPKs),inculuding p38 MAPK (p38) and p42/44 MAPK (p42/44) were found to phosphorylated. Migration of cells toward SPC was reduced in the presence of SB-203580, an inhibitor of p38, but not PD-98059, an inhibitor of p42/44, Pertussis toxin (PTX), aGi prptein inhibitor, induced an inhibitory effect on p38 phosphorylation and A7r5 cells migration. Myosin light chain (MLC) phosphorylation occurred after SPC stimulation with or without pretreatment with SB-203580 or PTX. The MLC kinase inhibitor ML -7 and the Rho kinase inhibitor Y-27632 inhibited MLC phosphorylation but only partially inhibited SPC-directed miglation. Complete inhibition was achieved with the addition of SB-203580. After SPC 1 mM stimulation, actin cytoskeleton formed thick bundles of actin filaments around the periphery of cells, and the cells were surrounded by elongated a– actin and b- actin, but magnapodia consisited exclusively of –actin. Such a remodeling of actin was reversed by addition of SB-203580 and PTX, but not ML-7 or Y-27632. Taken together, our biochemical and morphorogical data confirmed the regulation of actin remodeling and suggest that A7r5 cells migrate toward SPC, not only by an MLC phosphorylation-dependent pathway, but also by an MLC phosphorylation-independent pathway.

3-B-O16-5 一般演題（口頭）

# Comparative study of effects of NAd and PACAP on contractile and electrical activities in human colon.

Yoshihiko Kito[1], Masaaki Kurahashi[2]

*[1]Dept. Pharmacol. Fac. Med. Saga Univ., [2]Dept. Intern. Med. Div. Gastroenterol. Hepatol. Univ. Iowa*

We have previously reported that noradrenaline (NAd) inhibited mouse colonic motility via the α1A adrenergic receptor (α1A AR)-small conductance $Ca^{2+}$-activated $K^+$ (SK) channel signal pathway in PDGFRα$^+$ cells. In the present study, we studied the effects of NAd and pituitary adenylate cyclase-activating polypeptide (PACAP) on contractile and electrical activities of circular smooth muscle cells (CSMCs) in the human colon. NAd inhibited colonic contractions through binding to α1A AR. PACAP and maxadilan, a PACAP type 1 receptor (PAC1) agonist, also inhibited colonic contractions. The inhibitory effects of these drugs were sensitive to apamin, a blocker of SK channel. However, the latency of the responses to PACAP or maxadilan were significantly longer than NAd. Similar latency of the responses was also observed when we recorded apamin-sensitive membrane hyperpolarization evoked by NAd and maxadilan in colonic CSMCs intracellularly. These results suggest that PDGFRα$^+$ cells integrate inhibitory inputs from NAd and PACAP, leading to the activation of G protein coupled receptor-SK channel signal pathway in the human colon. NAd and PACAP may work together with different inhibitory time course to regulate colonic contractility during sustained stress.

3-B-O17-1 一般演題（口頭）

# Anticancer drug nilotinib-induced electrical, calcium, and mechanical alternans in human iPS cell-derived cardiomyocyte sheets

Hiroko Izumi-Nakaseko[1], Ryuichi Kambayashi[1], Yuko Sekino[2,3], Yasunari Kanda[4], Atsushi Sugiyama[1]

*[1]Dept. Pharmacol., Fac. Med., Toho Univ,[2]Grad. Sch. Agricultural and Life Sci., Univ. Tokyo,[3]Inst. Drug Discovery Innovation, [4]Div. Pharmacol., NIHS*

Purpose and Method: Nilotinib has been reported to clinically cause QTc prolongation. We observed that nilotinib at 1 μg/mL induced repolarization delay showing early afterdepolarization (EAD) with electrical alternans under electrical pacing in human-induced pluripotent stem cell-derived cardiomyocytes sheets. To characterize the alternans, we adopted field potential (FP), $Ca^{2+}$ imaging, and motion vector analyses.

Results: Nilotinib induced EADs in every excitation for the most part, but excitation without EAD showed up intermittently, and EADs were sometimes terminated by electrical pacing via the MEA system, which depended on the pacing cycle lengths. Alternate phenomena of FP duration (FPD) were observed with and without EAD, which also caused alternans in the conduction speed, contraction velocity and peak amplitude of Ca peak along with Ca transient duration. During electrical pacing, electrical excitations with EADs induced conduction delays upon the next one without EAD. Transient loss of EAD increased the conduction speed, contraction velocity and Ca peak amplitude of the cell sheets upon the next electrical excitation, indicating that the shorter FPD enhanced the recovery of Ca and Na channels from their inactivation state. Alternans was not observed when only action potentials without EAD or with EAD were sustained.

Conclusion: Intermittent loss of EAD would enhance electrical, calcium, and mechanical alternans of the next beat in the in vivo heart.

3-B-O17-2 一般演題（口頭）

# Analysis of cardiohemodynamic and electrophysiological effects of morphine using the halothane-anesthetized dogs

Ai Goto, Ryuichi Kambayashi, Hiroko Izumi-Nakaseko, Yoshinori Takei, Atsushi Sugiyama

*Dept. Pharmacol., Faculty. Med., Toho Univ.*

**Introduction:** While morphine has been used for treatment-resistant dyspnea in patients with end-stage heart failure, its information on in vivo cardiovascular profile remains limited.

**Methods:** Morphine was intravenously administered to the halothane-anesthetized dogs (n=4) in escalating doses of 0.1 followed by 1 mg/kg/10 min with respective 20 min observation periods under the monitoring of cardiohemodynamic and electrophysiological variables.

**Results:** The low dose hardly altered cardiovascular variables. The high dose reduced preload and afterload to the left ventricle for 5-15 min after the start of infusion, then decreased the left ventricular contractility along with the mean blood pressure for 10-30 min, and next suppressed the heart rate for 15-30 min. Morphine slowed the atrioventricular conduction and ventricular late repolarization, and prolonged the ventricular effective refractory period without altering the intraventricular conduction or ventricular early repolarization. A reverse-frequency-dependent delay of ventricular repolarization was confirmed.

**Conclusion:** Morphine could directly dilate the resistance and capacitance vessels, whereas it would attenuate the adrenergic tone followed by an increase of vagal tone. The reverse-frequency-dependent delay of ventricular repolarization by morphine along with the prolongation of late repolarization suggests hERG $K^+$ channel inhibition in vivo in spite of its large $IC_{50}$ value for hERG $K^+$ channel in vitro (>1 mM), indicating the presence of indirect mechanisms for its inhibition.

3-B-O17-3 一般演題（口頭）

# Effect of medetomidine, an anesthetic for animals, and dexmedetomidine, a sedative for humans, on thrombus formation.

Taiki Kano[1], Yui Suganuma[1], Kazuhisa Ikemoto[1], Chiho Ichinose[1], Toshiaki Mochizuki[2], Kazunao Kondo[1]

*[1]Dept. Pharmacol., Sch. Med., Fujita Health Univ., [2]Anesthesiology and Resuscitology, Fujita Health Univ. Okazaki Med. Ctr.*

Background

Medetomidine (MED) is used for animal experiment as mixed anesthesia (Medetomidine-Midazolam-Butorphanol: MMB). On the other hand, Dexmedetomidine (DEX), its enantiomer, is used for human sedation in the intensive care situation. It is unknown whether or not they may accelerate thrombogenesis via platelets $\alpha_2$-receptors stimulation and we evaluate their effect on platelet aggregation and thrombotic vessel occlusion using animal model.

Method

ICR mice (6-month-old males) were used for the experiments.

1) Blood was collected under pentobarbital anesthesia (80 mg/kg-i.p.), and collagen (0.8 μg/mL)-induced platelet aggregation was measured under DEX or MED supplementation.

2) Mice were injected with 1 μg/kg DEX or 0.75 mg/kg MED i.p., before pentobarbital 80mg/kg anesthesia, and p latelet aggregation was evaluated similarly as described above.

3) Vascular occlusion time was measured by ferric chloride (FeCl3)-induced thrombosis models under MMB or pentobarbital anesthesia.

Results

1) Platelet aggregation was enhanced by higher concentrations at 10 ng/mL DEX or 100 ng/mL MED supplement. The lower concentrations under 1 ng/mL DEX or 10 ng/mL MED did not affect aggregation.

2) 1 μg/kg MED i.p. enhanced platelet aggregation, while 0.75 mg/kg DEX did not.

3) Thrombotic occlusion time of mice femoral artery was shortened in MMB-anesthetized animals in comparison with pentobarbital-anesthetized ones.

Discussion

Clinical doses of DEX seem not affect thrombogenicity, while MED in MMB-animal anesthesia was revealed to enhance it.

3-B-O17-4 一般演題（口頭）

# Characterization of the cardiac safety pharmacological profile of anti-influenza drug peramivir using the isoflurane-anesthetized dog

Ryuichi Kambayashi, Ai Goto, Hiroko Izumi-Nakaseko, Yoshinori Takei, Atsushi Sugiyama

*Dept. Pharmacol., Faculty Med., Toho Univ.*

**Introduction:** Peramivir, anti-influenza drug, was reported to induce QT prolongation in some phase III studies. Such the effect on cardiac repolarization may have some potential to induce lethal ventricular arrhythmia, torsade de pointes (TdP). Accordingly, we characterized the cardiac safety pharmacological profile of peramivir by assessing ventricular and atrial electrophysiological actions in addition to cardiohemodynamic effects.

**Methods:** Peramivir in doses of 1 and 10 mg/kg/10 min (subtherapeutic and clinically-relevant doses, respectively) was intravenously administered to isoflurane-anesthetized dogs under the monitoring of electropharmacological variables (n=4).

**Results:** Peramivir decreased total peripheral vascular resistance, whereas it increased cardiac output and kept mean blood pressure at the basal control level. Meanwhile, peramivir prolonged QT interval/QTcV and $T_{peak}$-$T_{end}$ without altering J-$T_{peak}$c or intra-atrial, atrioventricular as well as intra-ventricular conduction. Peramivir also delayed ventricular repolarization and increased refractoriness at the same site, and tended to prolong terminal repolarization period. Peramivir prolonged atrial effective refractory period, of which extent was smaller than those of existing anti-atrial fibrillatory drugs; moreover, its atrial selectivity was lower among the drugs.

**Conclusion:** The clinical dose exposure of peramivir can develop a substrate for inducing TdP,but may not provide its trigger, suggesting that it would have the least potential for the onset of TdP.

3-B-O17-5 一般演題（口頭）

# Identification of a novel gene that regulates proliferation and lipid metabolism in lymphatic endothelial cells

Akira Sugiyama, Xinyi Liu, Tomohiro Shiiya, Yasuhiro Yoshimatsu, Masanori Hirashima

*Div. Pharmacol. Grad. Sch. Med. Dent. Sci. Niigata Univ.*

Developmental lymphatic vascular defects are a major cause of fetal nuchal edema, which is characterized by a subcutaneous accumulation of extracellular fluid in the fetal neck. Fetal nuchal edema is visualized as increased nuchal translucency by ultrasonography. It is found to be associated with chromosomal anomalies in about ten percent of clinical cases, whereas causative genes have not been elucidated in many clinical cases. In this study, we aimed to identify novel causative genes of fetal nuchal edema and lymphatic vascular defects through N-Ethyl-N-nitrosourea-induced mutagenesis screening in mice. This screening detected mouse embryos exhibiting both fetal nuchal edema and lymphatic vascular defect. Exome sequencing of the genomic DNA from these embryos revealed the candidate causative genes for fetal nuchal edema. Knockout of a candidate causative gene by CRISPR/Cas9 system in mice induced fetal nuchal edema and lymphatic vascular defects. Gene silencing by small interfering RNA in culture inhibited proliferation of lymphatic endothelial cells and also reduced mRNA expression of *HMGCR* and *FASN* encoding 3-hydroxy-3-methylglutaryl-coA reductase and fatty acid synthase, respectively. In conclusion, we identified a novel gene that regulates both proliferation and lipid metabolism in lymphatic endothelial cells.

3-B-O18-1 一般演題（口頭）

# Transient receptor potential melastatin 2 is involved in trinitrobenzene sulfonic acid-induced acute and chronic colitis-associated fibrosis progression in mice

Nakamoto Tomohiro[1], Kejiro Matsumoto[1,2], Hiroyuki Yasuda[1], Yasuo Mori[3], Shinichi Kato[1]

*[1]Lab. Pharmacol. Exp. Ther., Kyoto Pharm. Univ.,[2]Dept. Pathophysiol., Fac. Pharm. Sci., Doshisha Women's Coll., [3]Dept. of Synthetic Chem. and Biological Chem., Grad. Sch. of Eng., Kyoto Univ.*

Crohn's disease, a chronic and recurrent gastrointestinal disease, frequently causes intestinal fibrosis. Transient receptor potential melastatin 2 (TRPM2) belonging to TRP channel family is activated by reactive oxygen species. This study investigated the role of TRPM2 in acute colitis and chronic colitis-associated fibrosis progression. Acute colitis and chronic colitis-associated fibrosis were induced in TRPM2-deficient (KO) and wild-type (WT) mice through single and repeated intrarectal injections of trinitrobenzene sulfonic acid (TNBS). Bone marrow-derived macrophages (BMDMs) were created by M-CSF stimulation. In WT, a single TNBS injection induced acute colitis and upregulated inflammatory cytokines/chemokines, Th1 and Th17-related cytokines, and their transcription factors. In contrast, repeated TNBS injections induced chronic colitis-associated fibrosis and upregulation of fibrogenic factors, Th2-related cytokines, and its transcription factor. However, these increases were considerably suppressed in KO. Treating BMDMs with $H_2O_2$ increased inflammatory, Th1 and Th17-related cytokines expression, and JNK and ERK phosphorylation, but these responses were significantly less in KO than those in WT. These finding suggest that TRPM2 contributes to acute colitis progression via Th1/Th17-mediated immune responses. Furthermore, TRPM2 may be directly involved in colitis-associated fibrosis induction, likely due to the regulation of Th2/TGF-$\beta$1-mediated fibrogenesis in addition to a consequence of acute colitis progression.

3-B-O18-2 一般演題（口頭）

# Proteasome inhibitor attenuates the activation of hepatic stellate cell

Atsushi Umemura[1], Ayana Fujiwara[2], Anna Tanaka[2], Kazumi Iwata[1], Kikuko Amagase[2]

[1]*Kyoto Prefectural University of Medicine,* [2]*Ritsumeikan University*

Chronic liver disease progresses to cirrhosis and often develops into hepatocarcinoma. The final stage of the disease is fatal liver failure, and death from liver cancer is common. The development of treatments for chronic liver diseases has been a priority to prevent the progression to cirrhosis and the development of hepatocarcinoma. In fact, there are no approved drugs for nonalcoholic fatty liver disease (NAFLD) which represents the most common cause of chronic liver disease in many countries. It is now well-established that in NAFLD, mortality is highest with advanced fibrosis. Therefore, there is an urgent need to develop therapies that inhibit or ameliorate liver fibrosis.

Liver fibrosis is common pathological condition of chronic liver diseases caused by scarring due to excessive production and accumulation of extracellular matrix. Hepatic stellate cells, which are activated by liver injury and are a major source of collagen and other extracellular matrix, have attracted attention as a therapeutic target for liver fibrosis. We conducted a drug screening using human-derived hepatic stellate cells and found that several proteasome inhibitors including bortezomib and carfilzomib suppressed the function and proliferation of hepatic stellate cells. Furthermore, a 2$^{nd}$ generation proteasome inhibitor, carfilzomib also suppressed cell proliferation and decreased the production of extracellular matrix including *Collagen* and *Timp1* in hepatic stellate cells isolated from mouse liver. Currently, CCL4-induced liver fibrosis is being studied by using a mouse model.

Inhibiting the function and proliferation of hepatic stellate cells with proteasome inhibitors, especially carfilzomib, could potentially suppress and improve liver fibrosis and provide a new treatment widely applicable to chronic liver disease.

3-B-O18-3 一般演題（口頭）

# RAMP1 signaling attenuates diet-induced steatotic liver disease in mice

Yoshiya Ito[1,2], Kanako Hosono[1,2], Tomohiro Betto[3], Mina Tanabe[2], Atsushi Yamashita[2], Yu Kuroda[2], Mariko Kamata[1,2], Koh Hatanaka[1], Masataka Majima[4], Hideki Amano[1,2]

*[1]Dept. Pharm., Sch. Med., Kitasato Univ., [2]Dept. Mol. Pharm., Grad. Sch. Med., Kitasato Univ., [3]Dept. Gastroenterol, Sch. Med., Kitasato Univ., [4]Fac. Health & Med. Sci., Kanagawa Inst. Tech*

Objective: In recent years, most prevalent liver disease is metabolism dysfunction-associated steatotic liver disease (MASLD). MASLD-associated inflammation develops steatohepatitis, which further progresses to liver cirrhosis and liver cancer. Inflammation is regulated by the interaction between the nervous system and the immune system. We reported that a neuropeptide, calcitonin gene-related peptide CGRP acts on the CGRP receptor, receptor activity modulating protein 1 (RAMP1), to suppress inflammation. The objective of the present study examined whether RAMP1 signaling contributed to the progression of liver inflammation in MASLD.

Methods and Results: Male RAMP1 deficient ($RAMP1^{-/-}$) or wild-type (WT) mice were fed a normal diet (ND) or HFD for 12 weeks. HFD-fed $RAMP1^{-/-}$ mice showed heavier body weights than HFD-fed WT mice, which was associated with high levels of liver weights, ALT, total cholesterol, and glucose. HFD-fed $RAMP1^{-/-}$ mice had higher gene expression levels related to fibrosis including alpha SMA and collagen 1a1 and to inflammation including TNF and IL-1beta than HFD-fed WT mice. Flow cytometry analysis revealed that both genotypes fed with HFD decreased Kupffer cells (KCs). HFD-fed WT mice showed increased monocyte-derived KCs and monocyte-derived macrophages, while HFD-fed $RAMP1^{-/-}$ mice showed no changes in monocyte-derived KCs and macrophages.

Conclusions: These results suggested RAMP1 signaling deficiency enhanced diet-induced liver steatosis with liver inflammation and fibrosis.

3-B-O18-4 一般演題（口頭）

# Sympathetic nerves suppress the activation of resident macrophages in the gastrointestinal muscularis layer via beta adrenergic receptors

Tomo Fukuda, Noriyuki Kaji

*Lab.Vet.Pharmacol.,Sch.Vet.Med.,Azabu Univ.*

【Introduction】 Resident macrophages within gastrointestinal muscularis layer (MM$\phi$) are located near sympathetic nerves. However, the role of adrenergic signaling in regulating MM$\phi$ activation during inflammation remains unclear. This study aims to elucidate the the role of adrenergic receptors in MM$\phi$ activation.

【Methods】 The expression of adrenergic receptors mRNA in isolated MM$\phi$ was examined using RT-PCR. The J774.1 macrophage cell line was treated with LPS for 24 hours, either in the presence of adrenergic receptor agonists or antagonists. The production of nitric oxide (NO) was evaluated using the Griess method as an indicator for MM$\phi$ activation.

【Results】 MM$\phi$ expressed mRNA for $\alpha_1$, $\alpha_2$, $\beta_1$, and $\beta_2$ adrenergic receptors. Stimulation of J774.1 with LPS increased NO production. This increase in NO production was significantly reduced by both dobutamine and clenbuterol. Treatment with noradrenaline significantly decreased the LPS-induced NO production. Propranolol suppressed the inhibitory effect of noradrenaline on NO production. Additionally, both atenolol and butoxamine suppressed the noradrenaline-induced inhibitory effect on NO production.

【Conclusion】 Our results suggest that noradrenaline released from sympathetic nerves might suppress the inflammatory activation of MM$\phi$ via $\beta_1$ and $\beta_2$ adrenergic receptors.

3-B-O18-5 一般演題（口頭）

# Stratification and prediction of peg-IFN treatment efficacy in chronic hepatitis B patients

Naotoshi Nakamura[1], Kwangsu Kim[2], Shingo Iwami[1]

*[1]interdisciplinary Biology Laboratory, Graduate School of Science, Nagoya University,[2]Department of Scientific Computing, Pukyong National University*

The goal of antiviral treatment for chronic hepatitis B is to prevent disease progression by suppressing hepatitis activity; 48-week treatment with peg-IFN is used as first-line antiviral treatment for persistently infected individuals. However, because treatment efficacy is heterogeneous among individuals and treatment has side effects, it is desirable to be able to identify early which individuals would benefit from treatment. In this study, time series data on three serum biomarkers, HBV DNA, HBsAg and HBcrAg, which determine treatment response, were used to stratify the treatment efficacy of patients. First, a mathematical model describing the time-series dynamics of these biomarkers was constructed and parameters were estimated for each individual using non-linear mixed effects modeling. Clustering of patients based on these parameters showed that patients with high treatment efficacy were concentrated in a specific cluster. This cluster was characterized by lower biomarker levels at baseline compared to other clusters, with HBsAg and HBV DNA declining by more than 1 log10 during the first several weeks of treatment. The degree of decline in the amount of cccDNA remaining in hepatocytes was also greater. Therefore, a machine learning model was created to predict this cluster using random forest. The results showed that using both the initial biomarker levels at the start of treatment and the cumulative levels up to several weeks after treatment, it was possible to identify a group of patients with high treatment efficacy with sufficient accuracy. Other blood markers and patient background factors were also found to be associated with treatment response. Thus, dynamical systems phenotyping based on multivariable time-series biomarkers allows patient stratification and prediction of treatment efficacy. We expect that such a method could also be used to stratify patients with other diseases.

3-B-O19-1 一般演題（口頭）

# The effects of hypoxia-inducible factors on tumor macrophages in the tumor microenvironment and tumor immunity

Shinji Matsunaga, Ryo Hirakawa, Takujirou Homma, Kentarou Tokudome, Shuhei Tomita

*Dept.Pharmacol. Osaka Metro. Univ. Grad. Sch. of Med.*

The tumor tissue environment is considered hypoxic condition. The hypoxic condition inhibits hypoxia inducible factor (HIF) degradation and HIFs are constitutively active through prolylhydrosylase deactivation. Furthermore, in tumor cells, the activation of HIF-1a is considered as a tumor exacerbation factor that promotes proliferation, metastasis, and resistance to therapy in tumor cells. On the other hand, in immune cells, HIF-1α is involved in enhancing the inflammatory and immune response, activating T cells, and then acting as a tumor suppressor. These controversial things are not clear in vivo models. Therefore, it is needed to reveal whether upregulating HIFs level is beneficial for cancer therapy or not.

we have used Lewis lung carcinoma (LLC) syngeneic tumor mouse models and used macrophage-specific VHL knockout mice. Macrophage were collected from transplanted mice tumor and performed RNA-seq analysis. We explored genes that were highly expressed in tumor suppress macrophages and found candidate genes regulated by HIF. These candidate genes were evaluated and confirmed to be secreted from macrophages and inhibit tumor growth in vivo. These results suggest that upregulation of HIF-1 in macrophage could contribute to inhibit tumor growth.

3-B-O19-2 一般演題（口頭）

# Hornerin expressed in endothelial cells implicates in angiogenesis

Takayuki Okamoto[1], Yukiko Katsube[2], Mai Hattori[2], Haruki Usuda[1], Junichi Ota[2], Tetsuro Nikai[2], Koichiro Wada[1]

*[1]Dept. Pharmacol. Shimane Univ., [2]Dept. Anesthesiol. Shimane Univ.*

Thrombomodulin (TM) expressed on vascular endothelial cells plays an important role in activation of the anti-coagulant protein C pathway. TM interacts with multiple ligands and regulates several endothelial cell functions. Here, we found hornerin as a candidate protein interacting to TM on endothelial cell surface by mass spectrometry-based approach. It has reported that hornerin has similar features to filaggrin and implicates in the epidermal barrier function. However, the expression and role of hornerin in endothelial cells is still elusive.

In this study, we confirmed that hornerin protein and mRNA was expressed in endothelium of aorta and cultured endothelial cells by immunofluorescence staining, western blot, and RT-PCR. Lipopolysaccharide (LPS)-mediated sterile inflammation increased serum concentration of hornerin in mice, whereas reduced hornerin from cultured cell. Of note, TM knockdown cells emerged the reduction of hornerin on endothelial cell surface. Furthermore, HRNR knockdown impaired angiogenic tube formation of endothelial cells.

Our findings demonstrated that hornerin is expressed on vascular endothelial cells via the coordinating with TM. Therefore, endothelial hornerin might be an important component in the regulatory machinery of vascular inflammation and angiogenesis.

3-B-O19-3 一般演題（口頭）

# Development of anti-CD44 isoform-specific monoclonal antibodies

Suzuki Hiroyuki, Mika Kaneko, Yukinari Kato

*Dept. Antibody Drug Development, Tohoku University Grad. Sch. of Med.*

CD44 plays important roles in the tumor progression and has various isoforms, which are generated by the alternative splicing of CD44 mRNA. The mRNA of CD44 standard (CD44s) isoform is produced by constant region exons including the first five (1 to 5) and the last five (16 to 20). The mRNAs of CD44 variant (CD44v) isoform are produced by the assembling of variant exons (v1–v10) with the constant region exons of CD44s. CD44s and CD44v receive the post-translational modifications, such as *N*-glycosylation and *O*-glycosylation. Both CD44s and CD44v (pan-CD44) can attach to hyaluronic acid, which is important for cellular adhesion, homing, and motility. CD44v isoverexpressed in tumors and promotes tumor malignant progression through the binding to growth factors, and the acquisition of invasiveness, stemness, and drug resistance. These were mediated by the unique functions of the variant's exon-encoded region. Our group have established anti-CD44 monoclonal antibodies (mAbs) using the Cell-Based Immunization and Screening (CBIS) method. We would like to introduce the isoform-specific mAbs against CD44 and their application including flow cytometry and immunohistochemistry.

3-B-O19-4 一般演題（口頭）

# Probiotic derived heptelidic acid is cytotoxic in pediatric B-cell acute lymphoblastic leukemia

Konishi Hiroaki[1,3], Yuki Murakami[2,3], Koji Yamamoto[2], Chikage Yamamura[2], Noriko Satake[3], Mikihiro Fujiya[2]

*[1]Asahikawa Med Univ. Dept. of Gastro. Adv. Med. Sci., [2]Asahikawa Med Univ. Dept. of Med., [3]UC Davis. Dept. of Ped.*

Pediatric B-cell acute lymphoblastic leukemia (B-ALL) is known to be sensitive to chemotherapy. However, 15-20% of patients experience relapse, and survival rates after relapse can drop to 5-10%. We isolated heptelidic acid (HA) from the probiotic Aspergillus oryzae and demonstrated HA exerted antitumor functions against several cancers. In this study, we assessed the antitumor effects of HA in B-ALL. HA exhibited cytotoxicity in two B-ALL cell lines and three pediatric B-ALL patient samples, while sparing CD34+ hematopoietic stem cells (HSCs). GAPDH activity was measured, as HA can bind to GAPDH. GAPDH activity of HSCs was lower than B-ALL cells. The cytotoxic effect of HA was associated with the reduction of GAPDH activity. Oral daily administration of HA prolonged the survival of the B-ALL patient derived xenograft (PDX) mice. HA demonstrated synergistic effects when combined with chemotherapeutics, especially vincristin (VCR). The combination therapy of HA and VCR resulted in improved survival outcomes in the B-ALL PDX model compared to single-agent treatments. HA treatment caused G2/M arrest, and this was accompanied by increased cleavage of PARP.Moreover, the cytotoxicity of HA was attenuated by RIPK1 inhibition. In conclusion, HA is cytotoxic in B-ALL cells by disrupting glycolysis through the inhibition of GAPDH. The therapeutic efficacy of HA is enhanced when combined with chemotherapeutic agents. The underlying mechanisms of HA involve the induction of RIPK-1 mediated programmed cell death. This is the first study to demonstrate HA as a novel therapeutic agent for B-ALL.

3-B-O19-5 一般演題（口頭）

# Suppression of tumor spheroid formation by matrix metalloproteinase 19 (MMP19) in pyruvate dehydrogenase-E1β (PDH-E1β) knockdown (KD) cells

Yukino Kobayashi, Koh Nakayama

*Dep. of Pharmacol., Sch. of Med., Asahikawa Med. Univ.*

Breast cancer is the most common cancer type in woman worldwide. Recurrence of breast cancer is connected to poor prognosis, therefore novel treatments to prevent the recurrence are required. PDH generates acetyl-CoA from pyruvate and drives TCA cycle. PDH complex is composed of five subunits. In our previous study, knockdown of PDH-E1β subunit in breast cancer MDA-MB-231 cells reduced tumor formation in mice. In the present study, we used spheroid as an *in vitro* model to clarify the mechanism behind the tumor suppression in PDH-E1β KD cells. Wild type (WT) cells formed spheroid in three days, whereas PDH-E1β KD cells required seven days to form one. To investigate the effects of PDH-E1β on extracellular matrix (ECM) degradation, we examined the expression of MMPs, which are the main enzymes to degrade ECM. Expression of *MMP19* and *MMP28* were increased in PDH-E1β KD cells. Further, spheroid formation was rescued and formed in three days by the addition of MMP inhibitor. We have previously shown that CREB, a transcription factor, induces *MMP1*. Thus, we hypothesized that CREB is activated and induces *MMP19* and *MMP28* in PDH-E1β KD cells. Phosphorylation of CREB was increased in PDH-E1β KD cells, and PDH-E1β KD cells transfected with CREB siRNA also formed spheroid in three days. Knockdown of CREB decreased *MMP19* but not *MMP28* in PDH-E1β KD cells. Altogether, it is indicated that CREB induces the transcription of *MMP19*, and inhibit spheroid formation.

1-B-SS01-1 学生セッション（口頭）

# Nardilysin is involved in autoimmune hepatitis via T-cells

Shinya Yoshida[1,3], Hiroki Satooka[2], Kiyoto Nishi[1], Mikiko Ohno[1], Takako Hirata[2], Akira Andoh[1,3], Eiichiro Nishi[1]

*[1]Dept. of Pharmacol, Shiga Univ. of Med. Sci., [2]Dept. of Biol, Shiga Univ. of Med. Sci., [3]Dept. of Gas, Shiga Univ. of Med. Sci.*

Autoimmune hepatitis (AIH) is a refractory inflammatory disease that causes progressive liver damage. While it has been suggested that regulatory T cells (Treg) have a significant role on the pathophysiology of AIH, the precise mechanism how AIH is controlled by Treg is still elusive.

We have previously demonstrated that nardilysin (NRDC), a member of the M16 family of metalloendopeptidase, is involved in several inflammatory diseases (e.g. rheumatoid arthritis, non-alcoholic fatty liver) via the activation of TNF-$\alpha$. NRDC was also reported to be involved in antigen processing, suggesting that NRDC in immune cells may play a significant role in the pathogenesis of autoimmune diseases including AIH.

In this study, T cell-specific NRDC-deficient mice (CD4-CKO) and control mice (CONT) were intravenously injected with concanavalin A (ConA), which is a well-established model of acute AIH. Examination of liver histology and serum hepatic enzymes demonstrated that liver injury in CD4-CKO was significantly milder than that in CONT. Transcriptome analysis of splenic T cells revealed that the downstream genes of Foxp3, the master transcription factor of Tregs, were significantly altered in the CD4-CKO.

Consistently, the number of liver-infiltrated Treg was significantly increased in CD4CKO compared with CONT. Finally, naïve T cells isolated from CD4-CKO differentiate into Tregs more efficiently in vitro compared with CONT. These findings suggest that NRDC controls the pathogenesis of AIH via Treg.

1-B-SS01-2 学生セッション（口頭）

# Glutamate treatment abrogates 5-fluorouracil-induced mucoenteritis

Jonan Shizuka[1], Shinichi Kato[2], Kikuko Amagase[1]

*[1]Lab. of Pharmacol. & Pharmacother., Grad. Sch. of Pharmaceut. Sci, Ritsumeikan Univ.,[2]Dep. of Pharmacol. & Exp. Therap., Kyoto Pharmaceut. Univ.*

While 5-Fluorouracil (5-FU) is the widely used chemotherapeutic agent, it often causes mucoenteritis with severe diarrhea, nausea, vomiting, and body weight loss. These conditions are related with impaired quality of life of patients with cancer. Glutamate (Glu) is a nonessential amino acid and the most important energy source in the small intestine. In this study, we aimed to evaluate the role of Glu on the 5-FU-induced mucoenteritis. Mucoenteritis was induced in male C57B/6N mice by repeated administration of 5-FU (50 mg/kg, i.p.). Glu was administered as a pretreatment for 7 days before the initial treatment of 5-FU. Disease severity was assessed by histological and physiological analysis. Moreover, cell proliferation, apoptosis, and intestinal permeability were assessed using immunohistochemistry. The effect of Glu on 5-FU-induced cell injury was also examined in IEC-6, rat intestinal epithelial cell line. The pretreatment with Glu significantly suppressed the histological changes, impairment of cell proliferation, and apoptosis by 5-FU. While the expression of excitatory amino acid transporters (EAAT) was decreased on the ileum tissue damaged by 5-FU, Glu treatment maintained a high expression level of EAAT. These results suggest that Glu prevents 5-FU-induced mucoenteritis via enhancement of Glu transporters. Thus, Glu administration may have protective effects on 5-FU-induced mucoenteritis.

1-B-SS01-3 学生セッション（口頭）

# Involvement of NRF2 transcription activity in prostaglandin $E_2$-induced facilitation of the inhibitory effect of caffeine on hepatic stellate cell activation.

Watanabe Yuta[1], Momoka Yamaguchi[1], Naoki Dohi[1], Akira Ooka[1], Shin-ya Saito[1,2], Tomohisa Ishikawa[1]

*[1]Dept. Pharmacol., Grad. Sch. Integr. Pharmaceut. and Nutritional Sci., Univ. Shizuoka,[2]Lab. Drug Discovery and Pharmacol., Fac. Veterinary Med., Okayama Univ. Sci*

Hepatic stellate cells (HSCs) are known to play a central role in liver fibrosis (LF), and considered to be a target for LF therapy. We have previously reported that caffeine (CAF) suppresses HSC activation via inhibition of adenosine receptors, while also observing that prostaglandin $E_2$ ($PGE_2$) facilitates the inhibitory effect of CAF on HSC activation. However, the mechanism remains to be elucidated. In the present study, we aimed to elucidate the molecular mechanism of the inhibitory effect of $PGE_2$ and CAF co-treatment. Despite sharing a common pathway for cAMP, intracellular cAMP levels showed no direct correlation with the inhibitory effect of $PGE_2$ and CAF co-treatment on HSC activation. RNA-seq analysis identified the transcription factor *NRF2* as a candidate gene involved in the inhibitory effect of $PGE_2$ and CAF co-treatment on HSC activation. Reporter assays and immunostaining exhibited that the combination of $PGE_2$ and CAF resulted in a significant augmentation of NRF2 transcription activity and enhanced nuclear translocation of NRF2. These results suggest that $PGE_2$ facilitates the inhibitory effect of CAF on HSC activation by amplifying the transcriptional activity of NRF2.

1-B-SS01-4 学生セッション（口頭）

# Protective role of orphan G protein-coupled receptor GPR35 in the pathogenesis of dextran sulfate sodium-induced colitis in mice

Tokuyama Kouga[1], Toma Yagi[1], Momoka Bunya[1], Saeko Yamamoto[1], Hiroyuki Yasuda[1], Michiko Saito[2], Shinichi Kato[1]

*[1]Lab. Pharmacol. Exp. Ther., Kyoto Pharm. Univ.,[2]BioSci. Res., Kyoto Pharm. Univ.*

Orphan G protein coupled receptor GPR35 is highly expressed in gastrointestinal tracts. We previously reported that GPR35 plays a protective role in the pathogenesis of colitis via promoting tissue repair and healing, but the detail mechanism is not fully understood. The present study investigated the protective role of GPR35 in the pathogenesis of dextran sulfate sodium (DSS)-induced colitis, especially in relation to epithelial barrier functions and inflammatory responses. Colitis was induced in male GPR35-deficient (GPR35KO) and wild-type (WT) mice by DSS treatment for 7 days. For intestinal epithelial barrier functions, mucus secretion and tight/adherence junction protein expression were examined. For inflammatory responses, cytokine expression was examined in bone marrow-derived macrophages (BMDM). DSS treatment produced severe colitis accompanied by body weight loss and diarrhea/bloody stool, but the severity was significantly aggravated in GPR35KO mice compared with WT mice. There is no difference in epithelial barrier functions between both mice. In contrast, LPS-induced upregulation of cytokine expression significantly enhanced in BMDM obtained from GPR35KO mice compared with WT mice. Further, lysophosphatidic acid, a GPR35 agonist, suppressed LPS-induced cytokine expression in BM from WT mice but not GPR35KO mice. These results suggest that GPR35 plays a protective role in the pathogenesis of DSS-induced colitis. This response may be mediated by attenuation of inflammatory responses but not regulating epithelial barrier functions.

1-B-SS01-5 学生セッション（口頭）

# Exendin-4 dosing time-dependently affects hepatic circadian clock through GLP-1 receptors in the central nervous system

Pingping Xu, Jun-ichi Morishige, Zheng Jing, Naoto Nagata, Hitoshi Ando

*Dept. Cell. Mol. Funct. Analysis, Kanazawa Univ. Grad. Sch. Med. Sci.*

Aim To investigate the influence of dosing time and the role of glucagon-like peptide-1 receptor (GLP-1r) in the central nervous system (CNS) on the hepatic clock-modulating effect of exendin-4 (Exe-4). Methods Male B6 mice and CNS-specific GLP-1r knockout mice were maintained under a 12 h/12 h light/dark cycle, with 4-h time restricted feeding (TRF) or daytime-feeding (DF). Exe-4 or vehicle was administered repeatedly at the beginning of the active phase (ZT12) or rest phase (ZT0) for 4 or 5 days. After the last dosing, liver samples were collected every 6 h for measuring mRNA expression levels of the clock genes. Results The DF induced a substantial phase shift of the hepatic clock, and Exe-4 administered at ZT12 almost completely inhibited this effect of DF. On the other hand, Exe-4 administered at ZT0 had a minimal effect on the DF-induced phase shift, but enhanced the amplitude of hepatic clock. Under the TRF condition, Exe-4 did not affect the phase of the hepatic circadian clocks regardless of the dosing time. In the CNS-specific GLP-1r knockout mice, the counteracting effect of Exe-4 on DF-induced phase shift disappeared. Conclusion These results indicate that the effect of Exe-4 on the hepatic clock is dosing time-dependent and mediated by GLP-1r in CNS.

1-B-SS02-1 学生セッション（口頭）

# Effects of *Schisandra chinensis* on memory deficit induced by disuse syndrome

Yusho Ishii[1], Chihiro Tohda[2]

[1]*Section of Neuromedical Science, Institute of Natural Medicine, University of Toyama,* [2]*Section of Neuromedical Science, Institute of Natural Medicine, University of Toyama*

“Disuse syndrome” is the declining state of physical and mental functions due to physical inactivity, and it became more familiar probrem for the elderly in frailty as well as for young people living in “hikikomori". Our laboratory was the first to discover that skeletal muscle atrophy induced memory deficit in mice and its responsible myokine, hemopexin (Nagase T, Tohda C. 2021). We also identified other myokine which transferred to the central nervous system to promote axon elongation (Kodani A, et al. 2019). These studies suggest a relationship between muscle atrophy and brain function and the involvement of myokines in the phenomenone. The aim of this study was to explore effective drugs for the muscle atrophy-induced memory deficit.

We focused on *Schisandra chinensis* (SC), because many reports indicated that SC extract and its constituents increased skeletal muscle mass and muscle strength in mice and also humans by oral treatment. However, no studies investigated effects of SC on myokine-mediated regulation for cognitive function.

This study examined the effect of intramuscular injection of SC extract on memory deficit in a mouse model of disuse syndrome. To identify SC extract-induced myokines, atrophied skeletal muscle was isolated from mice and treated by SCextract ex vivo. Several candidates were obtained on silver stained PAGE. Identification and functional contribution of those proteins to memory are under investigation. We clarify the potential of SC extract and its molecular mechanism for disuse-elicited cognitive deficit.

1-B-SS02-2 学生セッション（口頭）

# Exploration of key molecules for diosgenin-induced axonal regeneration in the brain.

Tomoya Nagata, Ximeng Yang, Chihiro Tohda

*Institute of Natural Medicine, Section of Neuromedical Science, Univ. of Toyama*

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by deposition of Aβ. Our therapeutic strategy for AD is to regenerate neural circuits in the brain to recover memory function. We previously found diosgenin as a candidate drug for regenerating axons in the brain and improving memory impairment in AD model (5XFAD) mouse. In addition, diosgenin-driven axonal regeneration is mediated by changes in expression levels of several proteins (e.g. SPARC, Galectin-1) in neurons. In this study, we aimed to explore key candidate molecules that regulate the expression of these axonal regeneration-related proteins and clarify their contributions to axonal regeneration and memory recovery.

RNA-seq analysis revealed that 7-day treatment of diosgenin (1 μM) to hippocampal neurons (ddY mice, P7) drastically upregulated the expression of Rn7sk, one of the well-known transcription factors. Overexpression of Rn7sk significantly promoted axonal growth and upregulated the expression of SPARC in primary cultured hippocampal neurons, and also recovered memory deficits in 5XFAD mice. This study suggests that Rn7sk may be a responsible molecule for diosgenin-induced axonal regeneration, which proposes a novel therapeutic target for AD treatment.

1-B-SS02-3 学生セッション（口頭）

# Exploration of signaling molecules involved in the reversion of activated hepatic stellate cells utilizing structural modification of small molecule compounds

Ooka Akira[1,3], Momoka Yamaguchi[1], Yuzuki Nagasawa[2], Kenji Yamashita[2], Kaori Inamura[2], Makoto Inai[2], Shin-ya Saito[4], Yoshitaka Hamashima[2], Tomohisa Ishikawa[1]

*[1]Lab. Pharmacol., Sch. Pharmaceut. Sci., Univ. Shizuoka, [2]Lab. Synth. Org. Chem., Sch. Pharmaceut. Sci., Univ. Shizuoka, [3]JSPS DC1, [4]Fac. Vet. Med., Okayama Univ. Sci.*

Hepatic stellate cells (HSCs) are activated in response to liver injury and secrete huge amounts of collagen, the primary cause of liver fibrosis (LF). Thus, the regulation of trans-differentiation of HSCs, both activation of quiescent HSCs and reversion of activated HSCs, is crucial for therapeutic strategy for LF. However, few compounds have been reported to have such effects and no definitive therapy is available. Here, we elucidate the effect of DIF-1, a compound inhibiting HSC activation we previously reported, on activated HSC and LF mouse model. DIF-1 reduced the expression of type I collagen α 1 (Col1a1) and α-smooth muscle actin, markers of activated HSCs, even when treated after HSC activation. We further performed *in silico* analysis utilizing the relation between structural transition and HSC reversion effect of several DIF-1 analogs to identify molecular target of DIF-1. DIF-1 reduced the expression of activated HSC marker genes (*Acta2, Col1a1, Pdgfrb*), while it increased that of a quiescent HSC marker gene (*Lrat*) in thioacetamide-induced LF mouse model. Moreover, DIF-1 reduced the amount of collagen fiber in liver tissue. Taken together with our previous report, we propose that DIF-1 is a useful compound for LF treatment, acting on both quiescent and activated HSCs.

1-B-SS02-4 学生セッション（口頭）

# *Ligilactobacillus animalis* isolated from canine intestinal microbiota significantly inhibits the development of allergic diseases in a mouse model via direct enhancement of IL-10 production by dendritic cells

Ibuki Yasuda[1], Mao Kaneki[1], Masaki Nagane[2], Shiro Takeda[3], Jumpei Uchiyama[4], Tomoki Fukuyama[1]

*[1]Lab. of Pharmacol., Sch. of Vet. Med., Azabu Univ.,[2]Lab. of Biochem., Sch. of Vet. Med., Azabu Univ.,[3]Lab. of Food Sci., Sch. of Vet. Med., Azabu Univ.,[4]Dept. of Bacteriol., Grad. Sch. of Med. Dent. & Pharmaceut. Sci., Okayama Univ.*

【Background and Purpose】

We are focusing on the veterinary clinical application of *Ligilactobacillus animalis* isolated from healthy canine intestinal microbiota. Our previous findings have indicated that oral administration of *L. animalis* significantly inhibited the development of atopic dermatitis in a mouse model. The objective of this presentation is to clarify the relevance of IL-10 production from dendritic cells in the suppression of skin allergies by *L. animalis.*

【Methods】

*L. animalis* was cultured to $1\times10^9$ CFU/mL according to the established method (Cultured in MRS medium for 24 hours). A mouse-derived dendritic cell line (DC2.4 cells) was co-cultured with *L. animalis* viable bacteria ($1\times10^8$ CFU/mL) for 24 hours, and the levels of IL-10 and TNF-$\alpha$ in culture supernatant was measured by ELISA. Phosphorylation of p65, a key transcription factor in NF-$\kappa$B signaling, in dendritic cells 24 hours after co-culture with *L. animalis* was also detected by Western blotting.

【Results and Discussion】

Co-culture with *L. animalis* significantly increased the production of IL-10 and TNF-$\alpha$ compared to the untreated group. p65-phosphorylation was also significantly enhanced by treatment of *L. animalis* compared to the untreated group. Previous studies demonstrated that there is a direct or indirect relationship between stimulation of NF-$\kappa$B and IL-10 production. Our findings imply that pre-treatment of *L. animalis* activate the NF-$\kappa$B signaling and promote the IL-10 production from dendritic cells, which resulted in the inhibition of allergic diseases.

1-B-SS02-5 学生セッション（口頭）

# Preventive effects of orally administered arctigenin on neovascular age-related macular degeneration

Aimi Shirakawa[1], Hiroto Yasuda[1], Shinsuke Nakamura[1], Yuichi Takajo[2], Satoshi Inamasu[2], Satoshi Yomoda[2], Shimpei Watanabe[2], Masamitsu Shimazawa[1]

*[1]Dept. Biofunctional Evaluation., Gifu Pharmaceut. Univ., [2]Kracie Holdings, Ltd.*

Neovascular age-related macular degeneration (nAMD) is an ocular disease characterized by choroidal neovascularization (CNV). For nAMD treatment, the intravitreal injection of anti-vascular endothelial growth factor (VEGF) drugs is commonly used. However, it can be highly invasive and burdensome for nAMD patients. Arctigenin is a component of the herbal medicine burdock root and known to exhibit anti-tumor and vascular normalizing effects. Here, we investigated the effect of oral administration of arctigenin on CNV formation. The murine CNV model was created by laser photocoagulation and arctigenin at 100 mg/kg was orally administrated once a day. CNV area, vascular leakage and the endothelial cell proliferation were evaluated. Oral administration of arctigenin suppressed CNV formation and vascular leakage. In the CNV lesion, the number of proliferating endothelial cells was reduced in the arctigenin-treated group. Furthermore, the effects of arctigenin were confirmed using *in vitro* experimental systems using human retinal microvascular endothelial cells (HRMECs). Arctigenin at 30 μM attenuated VEGF-induced HRMECs proliferation and migration. These findings suggest that oral administration of arctigenin has beneficial effects on choroidal neovascularization of nAMD.

1-B-SS03-1 学生セッション（口頭）

# Pathophysiological role of the chloride channel ClC3 in pulmonary arterial hypertension.

Amano Taiki[1], Aya Yamamura[2], Moe Fujiwara[1], Rubii Kondo[1], Yoshiaki Suzuki[1], Hisao Yamamura[1]

*[1]Dept. Mol. Cell. Pharmacol., Grad. Sch. Pharmaceut. Sci., Nagoya City Univ., [2]Dept. Physiol., Aichi Med. Univ.*

Pulmonary arterial hypertension (PAH) causes chronical increase in pulmonary artery pressure rises due to pulmonary vasoconstriction and vascular remodeling. Although several PAH drugs have been recently developed, no curative treatment has yet been achieved. Sustained elevation of cytosolic $Ca^{2+}$ concentration ($[Ca^{2+}]_{cyt}$) is closely associated to PAH pathogenesis such as enhanced contraction and excessive proliferation of pulmonary artery smooth muscle cells (PASMCs). $[Ca^{2+}]_{cyt}$ is regulated by membrane potentials which are partly regulated by voltage-dependent $Cl^-$ channels. In the present study, the functional expression of voltage-dependent $Cl^-$ channels (ClC family) was examined using PASMCs from normal subjects and idiopathic PAH (IPAH) patients. Expression analysis showed that ClC3 channel expression was increased in IPAH-PASMCs compared to normal-PASMCs. Swelling-activated $Cl^-$ channel currents were larger in IPAH-PASMCs than in normal-PASMCs, which were attenuated by ClC3 siRNA. The growth of IPAH-PASMCs was inhibited by $Cl^-$ channel blockers, niflumic acid and DIDS. ClC3 siRNA also decreased the proliferation of IPAH-PASMCs. In conclusion, the expression of ClC3 channels was upregulated in IPAH-PASMCs, resulting in excessive cell proliferation, which contributes to the pathogenesis of PAH.

1-B-SS03-2 学生セッション（口頭）

# Prolonged exposure to coffee alters serotonin transporter expression in intestinal epithelial cells via DNA methylation

Satoshi Kikkawa[1], Emi Kiyota[2], Kana Harada[1], Shigeru Tanaka[1], Izumi Hide[1], Miki Bundo[2], Kazuya Iwamoto[2], Norio Sakai[1]

*[1]Dept. Mol. Pharmacological Neuroscience., Grad. Sch. Biomed. Health Sci., Hiroshima Univ.,[2]Dept. of Mol. Brain Sci., Grad. Sch. of Med. Sci., Kumamoto Univ.*

The majority of peripheral serotonin (5-HT) is produced in the digestive tract and plays a crucial role in controlling intestinal peristalsis and energy metabolism. The serotonin transporter (SERT), expressed in intestinal epithelial cells, regulates the available amount of 5-HT. Recently, a functional CpG site (CpG3) was identified in the SERT gene promoter region, and its expression may be subject to epigenetic regulation. Coffee is the most widely consumed beverage worldwide and exhibits a U- or J-shaped relationship with the risk of a variety of diseases. While caffeine has traditionally been recognized as a key component of coffee, the physiological activity of dietary polyphenols, which can alter DNA methylation patterns, has recently gained attention.

In this study, the impact of habitual excessive coffee consumption on intestinal SERT expression was investigated using the Caco-2 cell line, an intestinal epithelial cell model. The uptake of 5-HT by SERT and its mRNA levels significantly and dose-dependently decreased following exposure to regular coffee and decaffeinated coffee for 24 to 48 hours. On the other hand, exposure to caffeine did not affect SERT mRNA levels. Additionally, well-known compounds of coffee such as chlorogenic acid did not exhibit any effects. Finally, the influence of coffee on the CpG3 methylation state was evaluated using pyrosequencing. Coffee exposure induced hypermethylation at CpG3.

This study reports the potential of excessive coffee consumption to decrease intestinal SERT expression and disrupt peripheral 5-HT regulation.

1-B-SS03-3 学生セッション（口頭）

# Upregulation of T-type $Ca^{2+}$ channels expression following phenotype switch of mouse hepatic stellate cells.

Naoki Kawata, Rubii Kondo, Yoshiaki Suzuki, Hisao Yamamura

*Dept. Mol. Cell. Pharmacol., Grad. Sch. Pharmaceut. Sci., Nagoya City Univ.*

Hepatic stellate cells (HSCs) are largely involved in hepatic fibrosis associated with liver diseases such as non-alcoholic steatohepatitis. An increase in cytosolic [$Ca^{2+}$] in HSCs facilitates hepatic fibrosis, however, the regulatory mechanism is unclear. T-type voltage-dependent $Ca^{2+}$ channels (T-VDCCs) contribute to neuronal transmission and cardiac rhythm in excitable cells. Recently, the pathophysiological roles of T-VDCCs in non-excitable cells such as cancer cells and immune cells have been focused. Under whole-cell patch-clamp configurations, transient inward currents were observed in mouse activated HSCs, but not in quiescent HSCs. Quantitative real-time PCR analysis revealed that the mRNA expression of T-VDCCs was significantly higher in activated HSCs than in quiescent HSCs. The viability of activated HSCs was significantly reduced by the treatment with T-VDCC inhibitors. These results suggest that the upregulation of T-VDCC expression in activated HSCs contributes to the regulation of $Ca^{2+}$ signaling and cell proliferation. This study may contribute to the comprehensive understanding of HSC functions in hepatic fibrosis.

1-B-SS03-4 学生セッション（口頭）

# Characterization of TRPM2 in canine peripheral blood mononuclear cells

Ueda Naoto, Takuya Yamaguchi, Jun Yamazaki

*Lab. Vet. Pharmacol., Dept. Vet. Med., Coll. of Biores. Sci., Nihon Univ.*

Transient receptor potential melastatin 2 (TRPM2) is a $Ca^{2+}$-permeable, non-selective cation channel that is activated by oxidative stress such as reactive oxygen species (ROS) and pyridine nucleotides such as adenosine diphosphate (ADP) ribose. TRPM2 channels are widely expressed in leukocytes, and TRPM2-mediated ROS-sensitive $Ca^{2+}$ signaling play a crucial role in a number of cellular processes and functions. In this study, transcript of TRPM2 was cloned from canine peripheral blood mononuclear cells (PBMCs) and some of functional properties of canine TRPM2 were analyzed *in vitro*. In canine *TRPM2* sequence, the basic structure of TRP channel and the binding sites for ADP ribose in MHR1/2 and NUDT9-H domains were conserved. We analyzed the reactivity of heterologously expressed canine TRPM2 to hydrogen peroxide ($H_2O_2$) in $Ca^{2+}$-imaging experiments. Canine TRPM2 was activated by $H_2O_2$ in a concentration-dependent manner. This reaction was inhibited by perfusion of TRPM2 inhibitor 2-APB or $Ca^{2+}$-free extracellular solution. Next, canine PBMCs were incubated with $H_2O_2$ and were subjected to RT-qPCR analysis. The expression of an anti-inflammatory cytokine *IL-10* was not enhanced, but the expression of inflammatory cytokines *IL-1β*, *TNFα*, and *IL-8 (CXCL8)* were tended to be enhanced after incubation with $H_2O_2$. Our results suggest that TRPM2 plays an important role in activation of canine leukocytes, and that TRPM2 is a possible target for regulation of leukocytes functions.

**1-B-SS03-5** 学生セッション（口頭）

# Vibrational spectroscopy study of chemical interaction between κ-opioid receptor (KOR) and ligands having morphinan structure

Ryo Nishikawa[1], Kota Katayama[1,2], Seiya Iwata[1], Ryoji Suno[3], Chiyo Suno[3], Takuya Kobayashi[3], Hideki Kandori[1,2]

*[1]Grad. Sch. of Engineering, Nagoya Inst. of Tech., [2]OptoBio Tech. Res. Ctr, Nagoya Inst. of Tech.,[3]Dept. of Med., Kansai Med. Univ.*

Opioid receptors (ORs) belong to a member of GPCRs and are receptive to compounds that exert analgesic effects, such as morphine. Many opioid ligands contain a morphinan structure, and their pharmacological activity (efficacy) varies, including agonists and antagonists. It is important in drug discovery to elucidate the binding mechanism of ligands to ORs and the relationship between their efficacy. Here, we applied vibrational spectroscopy-based GPCR-ligand interaction studies to κ-opioid receptor (KOR) to elucidate differences in the binding mechanism of various ligands to the receptor, which share a common morphinan structure but exhibit different efficacy. The agonist binding spectra showed the spectral down-shift in amide-I band reflecting to weakening the hydrogen bond between C=O and N-H pairs of peptide backbone, whereas the antagonist bound spectra showed the opposite change, which indicates the different conformational changes that occur between an agonist and antagonist binding to KOR. Furthermore, differences in the vibrational bands derived from functional group of amino acids were also observed for different ligand efficacy. The mechanism by which differences in pharmacological efficacy arise from a common morphinan structure will be discussed, based on protein backbone and functional group of amino acid changes.

**1-B-SS04-1** 学生セッション（口頭）

# Therapeutic effects of drugs developed for CFTR mutations in Caucasians on a Japanese Q98R CFTR mutation

Kimijima Rio[1], Yuzuki Takahasi[1], Noa Nakajima[1], Yuuka Saitou[1], Kana Kobayasi[1], Saki Uematu[1], Hikaru Shoma[1], Yoshiro Shoma[1], Kanako Nakao[1], Hiroshi Nakagawa[2], Ritsuko Imai[2]

*[1]Div Mol Ther, Grad Sch Pharm, IUHW, Japan,[2]Dept Biol Chem, Coll Biosci Biotech, Chubu Univ, Kasugai, Japan)*

Cystic fibrosis (CF) is an autosomal recessive disease in which mutations in the CFTR gene cause various symptoms through its channel malfunction. Among over 1900 gene mutations, the most common mutation is ⊿F508 which causes a trafficking defect of CFTR to plasma membrane (classified as 'class II' ). CF is quite rare in Japanese and listed in 'Intractable Diseases' by MHLW, Japan. Q98R mutation is the third most frequent disease associated mutation found in Japanese CF patients following H1085R and L441P. The Q98R mutation is in the class II same as H1085R, L441P and ⊿F508 mutations.

Some pharmaceutical companies have been developing several expression correctors, e.g., lumacaftor, galicaftor, tezacaftor and elexacaftor, and channel function potentiators, e.g., ivacaftor, for ⊿F508 mutation.

In this study we characterized the effects of the Q98R mutation on CFTR processing and investigated the effects of the Caucasian CF drugs on the expressions and functions of Q98R-CFTR.

From our *in vitro* data, it is suggested that Orkambi, a combination of lumacaftor and ivacaftor, is a potential candidate in the pharmaceutical therapy for Japanese CF patients with the Q98R CFTR mutation.

1-B-SS04-2 学生セッション（口頭）

# Rat TRPV1 and TRPA1 can constitute functional heterotetoramer.

Aya Kondo[1,2], Naoki Kitamura[2], Ken-ichi OTSUGURO[1]

*[1]Lab. of Pharmacol., Dept. of Basic Vet. Sci., Fac. of Vet. Med., Hokkaido Univ.,[2]Lab. of Vet Physiol., Dept. of Vet. Med., Fac. of Agr., Tottori Univ.*

Background: The non-selective cation channels TRPV1 (V1) and TRPA1 (A1) are respond to nociceptive stimulus and co-expressed in the dorsal root ganglia (DRG). Capsaicin, a V1 agonist, binds to the transmembrane region of V1, and allyl isothiocyanate (AITC), an A1 agonist, binds to the N-terminus of A1, and activates these channels. It has been suggested that V1 and A1 form a functional heterotetramer in native cells, but the details are unknown. We therefore created their artificial heterotetramers and analyzed whether V1 and A1 could form a functional heterotetoramer.

Methods: A1 and V1 were cloned from rat cDNA, and the A1::V1 tandem(A1::V1) was created by linking them with a linker. We also created a tandem of ΔN A1 and V1 (ΔN A1::V1), in which the N-terminus of A1 was shortened. These channels were expressed in HEK293 cells, and the responses of these channels to agonists were examined using the whole-cell patch clamp technique.

Results/Discussion: A1::V1 responded to both capsaicin and AITC. To investigate the contribution of A1 N-terminus to the agonist responsiveness of the A1::V1, we analyzed the function of ΔN A1::V1. ΔN340 A1::V1 (lack of 1st to 8th N-terminal ankyrin domain of A1) was found to be not responsive to both agonists. On the other hand, the ΔN308 A1::V1 (lack of 1st to 7th ankyrin domain of A1) responded to both agonists, suggesting that, the 8th N-terminal ankyrin domain of A1 is required for agonist responsiveness of the A1::V1.

These results suggest that TRPV1 and TRPA1 constitute heterotetramer and function as ion channels in native cells.

1-B-SS04-4 学生セッション（口頭）

# Vinculin-talin pre-complexes flow over mature focal adhesions without tension

Ying Liu[1], Sawako Yamashiro[1,2], Naoki Watanabe[1,2]

*[1]Lab. of Single-molecule Cell Biology, Kyoto Univ. Grad. Sch. Of Biostudies, [2]Dept. of Pharmacology, Kyoto University Faculty of Medicine*

Focal adhesions (FAs) are integrin-based, multiprotein structures that form links between the intracellular actin cytoskeleton and extracellular matrix (ECM). Vinculin is a major FA component and has been proposed to stabilize FAs in a force-dependent manner. While it is known that interaction of vinculin with F-actin is critical for vinculin's function, how vinculin associates with dynamic actin networks has remained unclear. At the cell periphery, the retrograde actin flow (a continuous centripetal movement of the actin network) is widely observed in adherent cells. By using Sigle-Molecule Speckle (SiMS) microscopy, we found that vinculin in lamellipodia exhibits retrograde flow-associated motion. To clarify how vinculin interacts with the lamellipodial actin network, we examined molecular motions of wild-type vinculin and three kinds of vinculin mutants: constitutively active, lacking F-actin binding, and weak talin binding mutants, respectively. We also observed that vinculin on matured FAs observed by SiMS microscopy exhibits both flowing and stationary fractions. Our findings suggest the following three points: (1) vinculin associates with the lamellipodial actin network mainly via binding to talin. (2) the vinculin-talin complex moves through actin retrograde flow over mature FAs without linkage between F-actin and integrins. (3) Vinculin-talin may form a pre-bound complex in a force-independent manner. These findings, employing direct observation of vinculin and actin at the molecular level, provide new insights into the molecular mechanisms of focal adhesion dynamics.

1-B-SS04-5 学生セッション（口頭）

# Development of a rapid and universal method to determine GPCR structures

Kojima Asato[1], Toshiki Matsui[1], Naoya Kobayashi[2], Masahiro Fukuda[1], Nakamura Seiwa[1], Kouki Kawakami[1], Kazuhiro Kobayashi[1], Hideaki Kato[1]

*[1]Arts and Sci., The Univ. of Tokyo,[2]Mater. Sci., NAIST*

More than 30% of drugs exert their effects by modulating the activity of G protein-coupled receptors (GPCRs) as agonists or antagonists. To understand the working mechanism of drugs and design new ones, the 3D structural information on therapeutic target proteins is crucial. However, every previous approach to determine the structures of GPCRs requires time-consuming experimental screenings of the expression construct for each target. This process significantly hinders high-throughput structural analysis of GPCRs. Moreover, there is no universal strategy for cryo-EM analysis of GPCRs in both agonist- and antagonist-bound forms.

Here, I present a new method for rapid cryo-EM structure determination of GPCRs, called NOAH (NOvel AI-assisted High-throughput construct screening for structural analysis). NOAH is a program that automatically generates the expression constructs of soluble protein-fused GPCRs suitable for cryo-EM analysis. By employing the NOAH pipeline, we can skip the process of experimental screening, saving a significant amount of time and resources on the project. As a proof-of-concept experiment, I applied this method to three GPCRs and determined not only the antagonist-bound structures but also an agonist-bound structure, demonstrating NOAH's potential to facilitate GPCR structural biology and drug discovery.

1-B-SS05-1 学生セッション（口頭）

# Age-related changes in immune function and pathogenesis in a mouse model of asthma

Mao Kaneki[1], Chiharu Ohira[1], Mana Ichikawa[1], Yoshiichi Takagi[2], Tomoki Fukuyama[1]

[1]*Lab. of Vet. Pharmacol., Sch. of Vet. Med., Azabu Univ.,*[2]*Japan SLC, Inc.*

Aging has been progressing rapidly worldwide. Therefore, it is important to investigate age-related differences in disease pathogenesis to develop age-appropriate treatment plan and drug regimen. In this study, we investigated age-related changes in immune function and pathogenesis in a mouse model of asthma. We compared the quantitative and functional changes of various immune cells in untreated or asthma-induced mice between 10-weeks-old (normal group) and 80-weeks-old (aging group) C57BL/6N mice. A mouse model of asthma was generated by repetitive intranasal administration of *Dermatophagoides farina*. In untreated mice, naive CD4 and CD8 T cells were significantly decreased in the aging group compared with the normal group, whereas effector CD4 and CD8 T cells and regulatory T cells were significantly increased in the aging group compared with the normal group. In a mouse model of asthma, the pathogenesis of asthma was significantly attenuated compared with the normal group based on the result of percutaneous arterial oxygen saturation ($SpO_2$). In addition, the aging group showed a significant decrease in type 2 innate lymphocytes and eosinophil counts. Our findings indicate that significant downregulation of immune function in the aging group attenuated the pathogenesis of asthma.

1-B-SS05-2 学生セッション（口頭）

# TRPV4 protects OVA-induced food allergy in mice via maintaining colonic epithelial barrier functions

Murayama Yuki[1], Kenjiro Matsumoto[1,2], Hiroyuki Yasuda[1], Shinichi Kato[1]

*[1]Lab. Pharmacol. Exp. Ther., Kyoto Pharm. Univ.,[2]Dept. Pathophysiol., Fac. Pharm. Sci., Doshisha Women's Coll.*

The prevalence of food allergy has increased worldwide but the pathogenesis remains undefined and effective treatments has not been established. Transient receptor potential vanilloid 4 (TRPV4), a mechanosensitive nonselective cation channel, is mainly expressed in epithelium of various organs. The present study investigated the role of TRPV4 in the pathogenesis of ovalbumin (OVA)-induced food allergy in mice. TRPV4 was mostly expressed in colonic epithelium. Repeated oral OVA challenge after sensitization induced food allergy, characterized by systemic allergic symptoms, diarrhea, upregulation of Th2-cytokines such as IL-4, IL-5, IL-13, and increase in serum OVA-specific antibodies, but all these responses were significantly augmented in TRPV4-deficient (TRPV4KO) mice compared with wild-type (WT) mice. Infiltration of CD11c-, CD117-, CD4-, and CD170-positive cells in the colon with OVA-induced food allergy was also enhanced in TRPV4KO mice compared with WT mice. Intestinal permeability determined by the FITC-dextran method was significantly increased in TRPV4KO mice compared with WT mice in normal and OVA-induced food allergy. Furthermore, the expression of adherence junction protein E-cadherin, tight junction protein claudin-3 and occludin in the colon was significantly lower in TRPV4KO mice than WT mice in normal and food allergy. These results suggest that epithelial TRPV4 protects OVA-induced food allergy. This response may be accounted for by suppressing the penetration of allergens via maintaining epithelial barrier functions.

1-B-SS05-3 学生セッション（口頭）

# Potential of Beta Caryophyllene (BCP) as the treatment for allergic responses through CB2 receptor activation

Hosoki Haruka[1], Chihiro Nozaki[2], Toru Asahi[1,3]

*[1]Waseda Univ. Grad. Sch. of Adv. Sci. & Eng.,[2]Waseda Univ. Global ctr. for Sci. and Eng. Major in Biosci.,[3]Waseda Univ. Res. Org. for Nano & Life Innov.*

Beta caryophyllene (BCP) is one of the sesquiterpenes found abundantly in cannabis as well as other plants such as clove, rosemary, black pepper, and lavender. Past studies have shown this terpene has antioxidant and anti-inflammatory effects, which is believed to be mediated by cannabinoid receptor type 2 (CB2). Recent study showed the attenuation of aortic inflammation by BCP inhalation, which is abolished by the treatment of CB2 antagonist.
CB2 receptor is the Gi/Go type GPCR majorly expressed on the immune-related cells throughout the body. Thus, CB2 has been reported to act as a regulator in the immune system, but not many researches has been conducted to see the therapeutic possibility of CB2 to treat e.g. autoimmune disease by their immune suppression properties. However, since BCP showed inflammatory suppression effect by inhalation, we expected that BCP might be able to attenuate other inflammatory responses, such as allergic diseases through CB2-mediated immune suppression. We therefore examined whether CB2 can suppress allergic responses, particularly by acute inhalation of BCP. The BCP inhalation attenuated sneezing and nose scratch in nasal allergy model mice. Further, the immune cell population of CB2-deficient mice shifted similarly to that of wild-type mice after BCP administration. Altogether, we propose that BCP could be a potential substance to cure allergic diseases.

# Treatment duration-dependent efficiency of quercetin in mast cell degranulation and transcriptome landscape

Matsuo Mana

*Department of Pharmacology, Ehime University Graduate School of Medicine, Tohon, Ehime, Japan*

Background: Quercetin inhibits the histamine release from activated mast cells. Our previous study revealed that the exposure of mast cells to quercetin for 24 hours resulted in an increased release of β-hexosaminidase. Here, we investigated the treatment duration-dependent efficiencies of quercetin on the activations of mast cells.
Methods: Rat basophilic leukemia cells (RBL-2H3 cells) were pre-treated with 1μM quercetin for 0 (control), 1 and 24 hours and the degranulation of mast cells was evaluated. A comprehensive transcriptome analysis was also performed in 1h-, 12h-, and 24h-quercetin-treated RBL-2H3 cells.
Results: A inhibitory effect of quercetin was confirmed in quercetin-treated mast cells with a pre-treatment duration of 1 h. On the other hand, an increase in the activity of mast cells was observed in quercetin-treated mast cells with 24 h pre-treatment period in a dose-dependent manner, compared with those in control cells. The difference in the expression of genes revealed a relationship between the pre-treatment duration of quercetin and cellular activation, such as, but not limited to calcium signaling pathway and Fc ε RI signaling pathway.
Conclusions: A diversity of functional responses of cultured mast cells to quercetin treatment was observed in the current study. Further study is required for elucidation of treatment duration-dependent efficacy in systemic response of allergic models.

1-B-SS05-5 学生セッション（口頭）

# Dasatinib suppresses particulate-induced pyroptosis and acute lung inflammation

Yixi Pan[1], Naoki Takemura[1], Tatsuya Saitoh[1,2,3]

*[1]Dept. Bioresp. Reg., Grad. Sch. Pharm. Sci., Osaka Univ., [2]Global Ctr., Med. Eng. Inform., Osaka Univ., [3]CiDER, Osaka Univ.*

Irritating particulates like PM2.5 cause inflammatory diseases. Such particulates cause phagolysosomal dysfunction of immune cells, resulting in the activation of the Nod-like receptor family pyrin domain containing 3 (NLRP3) inflammasome, an immune complex that induces cell death accompanied by the release of inflammatory mediators, namely pyroptosis. However, targeting NLRP3 inflammasome-associated responses is insufficient in treating relevant inflammatory diseases, as several particulates like silica particle (SP) also induce NLRP3-independent cell death and release of the inflammatory mediators like interleukin-1(IL-1) α. Therefore, drugs suppressing particulate-induced NLRP3-independent pyroptosis are warranted. In this study, we screened the compounds that can inhibit SP-induced cell death and IL-1α release using a high-content imaging-based system. As a result, we found that several Src family kinase (SFK) inhibitors, including dasatinib, effectively suppressed particulate-induced cell death and IL-1α release. Dasatinib reduced SP-induced phagolysosomal dysfunction. Moreover, dasatinib treatment suppressed the increase in IL-1α levels, and neutrophil count in SP-induced NLRP3-independent acute lung inflammation. In conclusion, dasatinib can inhibit particulate-induced pyroptosis by suppressing the phagolysosomal dysfunction and can be used to treat the relevant disease.

1-B-SS06-1 学生セッション（口頭）

# The role of germline mitochondrial DNA mutation in macrophages

Katsuki Wakayama[1], Chimere Ezuma[2], Keith Mcconn[2], Masanori Yoshizumi[1], Augustine Choi[2], Kiichi Nakahira[1,2]

*[1]Nara Medical University, Pharmacology, [2]Weill Cornell Medicine*

Accumulation of mutated mitochondrial DNA (mtDNA) results in mitochondrial dysfunction. We have previously reported that accumulation of mtDNA mutation increases inflammasome-mediated innate immune response in vitro and in vivo. Emerging evidences suggest that accumulation of maternally-transmitted mtDNA (m-mtDNA) mutation contributes to aggravating ageing. Meanwhile it remains unclear whether m-mtDNA mutation affects immune function in offspring mice. We generated a series of inbred mutant mice by intercrossing of mice heterozygous for the mtDNA mutator allele (PolgAwt/mut), which can generate mice harboring m-mtDNA (PolgAwt1). Bone marrow-derived macrophages (BMDM) from the first generation (N1) of PolgAwt1 (PolgAwt1-N1) display ~1.4 times more mtDNA mutation than cells from the control mice (Polgwt0-N1). However, inflammasome activation in BMDM was comparable between the two groups. There were no significant differences on the number of mtDNA mutation as well as inflammasome activation between cells from PolgAwt1-N2 and PolgAwt0-N2. Although the number of mtDNA mutation in BMDM from PolgAwt1-N3 was comparable to PolgAwt0-N3, inflammasome activation was further increased in BMDM from PolgAwt1-N3, compared to PolgAwt0-N3. These results suggest that m-mtDNA mutation may have an immunomodulatory effect to offspring.

1-B-SS06-2 学生セッション（口頭）

# Enhancement of wound healing by Atmospheric-Pressure Plasma exposure in a mouse model of thermal injuries

Chiharu Ohira[1], Mao Kaneki[1], Chizuki Usui[1], Masashi Nakamura[2], Kenichiro Miyasato[2], Yu Nagahara[2], Hideaki Kai[2], Eiji Miyamoto[2], Tomoki Fukuyama[1]

*[1]Lab. of Pharmacol., Sch. of Vet. Med., Azabu Univ.,[2]Sekisui Chemical Co., Ltd.*

Atmospheric-Pressure Plasma devices with an operational heat close to body temperature have received considerable attention due to their great potential for a variety of biomedical applications, such as acute and chronic wound healing, and regeneration of damaged tissues. Previous studies indicate that reactive oxygen species (ROS)/reactive nitrogen species (RNS) signals indirectly generated by Atmospheric-Pressure Plasma exposure have a positive effect on wound healing. The objective of this study is to investigate the wound healing effects of Atmospheric-Pressure Plasma in a mouse model of thermal injury. Thermal injuries are a major public health problem and cause severe physiological stress. In this project, a mouse model of second-degree burn wound was generated with a solid Al bar (41 g) which preheated in boiling water (80°C) for longer than 1 min on skin in four sites of male C57/BL6 mice for 30 s. The treatments of control gas or Atmospheric-Pressure Plasma were conducted for 15~60 sec for continuous 5 days. Atmospheric-Pressure Plasma was generated by Pidi® (Sekisui Chemical Co., Ltd.) which is the world's first therapeutic device for animals with gingivitis and halitosis using nitrogen plasma technology. Analysis will be performed on clinical signs, histological evaluations, and gene expression analysis.

1-B-SS06-3 学生セッション（口頭）

# Mitochondrial dysfunction exacerbates inflammatory responses in primary cultured chondrocytes

Hatsune Motonari[1], Yuka Tanaka[2], Yoki Nakamura[1], Kazue Nakashima[1], Norimitsu Morioka[1]

*[1]Dept. Pharmacol., Grad. Sch. Biomed. Health Sci., Hiroshima Univ., [2]Sch. Pharmaceu. Sci., Hiroshima Univ.*

Osteoarthritis (OA) is a chronic degenerative disease associated with inflammation and degradation of articular cartilage and is typically considered an age-related disease. In OA patients, persisting pain reduces quality of life. However, the mechanism underlying OA development remain unclear. Recent studies have reported that mitochondrial dysfunction occurs with aging and might contribute to aggravation of inflammation. Hence, we examined whether mitochondrial dysfunction has effects on inflammatory responses in chondrocytes. Primary cultured chondrocytes were prepared from knee articular cartilage of neonatal Wistar rats. Chondrocytes were co-treated with rotenone (Rote), an electron transport complex I inhibitor, and interleukin (IL)-1$\beta$. Co-treatment of cultured chondrocytes with Rote and IL-1$\beta$ potentiated the expression of inflammatory mediators such as matrix metalloproteinase 3, IL-6, and inducible nitric oxide synthase compared to each treatment alone. The potentiation was suppressed by blockade of transforming growth factor beta-activated kinase 1 (TAK1), c-Jun N- terminal kinase (JNK), or nuclear factor-$\kappa$B (NF-$\kappa$B). These results suggest that mitochondrial dysfunction potentiates inflammatory responses via the TAK1/JNK/NF-$\kappa$B pathway in chondrocytes. These responses might contribute to the induction of OA pathogenesis.

1-B-SS06-4 学生セッション（口頭）

# Administration of 5,6-DiHETE attenuated allergic inflammation in murine conjunctiva.

Shinya Takenouchi[1], Tomoka Suzuki[1], Nanae Nagata[1], Misato Kida[1], Takahisa Murata[1,2,3]

[1]*Dept. Anim. Radiol. Tokyo Univ.,*[2]*Dept. Vet. Pharmacol. Tokyo Univ.,*[3]*Dept. Food Anim. Syst. Tokyo Univ.*

Allergic conjunctivitis (AC) causes eyelid swelling, redness, tearing and itching when mast cells release mediators such as histamine (His). We reported 5,6-dihydroxy-8Z,11Z,14Z,17Z-eicosatetraenoic acid (5,6-DiHETE), metabolite of eicosapentaenoic acid, suppressed His-induced inflammation. We investigated the therapeutic effect of 5,6-DiHETE on AC.

Mice were injected i.p. with ragweed pollen (RW) on day 0 and 5 and by eye drop on day 10-14 to induce AC. On day 14, symptoms were scored based on swelling, redness and tearing. In AC, symptom score and tear volume increased 30 minutes after RW. Histological analysis revealed mast cell degranulation and eosinophil infiltration. 300 μg/kg 5,6-DiHETE i.p. just before RW suppressed the increase in symptom score, tearing and histological change. 1 μg 5,6-DiHETE by eye drop also suppressed these symptoms.

To reveal the mechanism, we evaluated the effect on mast cell degranulation *in vitro*. 1 μM 5,6-DiHETE 15 minutes before antigen treatment suppressed degranulation. We further evaluated the effect on vascular hyperpermeability. 300 μg/kg 5,6-DiHETE i.p. suppressed His-induced tearing and leakage of i.v. injected dye. Orally 600 μg/kg 5,6-DiHETE also suppressed tearing.

In conclusion, 5,6-DiHETE inhibited mast cell degranulation, vascular hyperpermeability and suppressed murine AC.

1-B-SS06-5 学生セッション（口頭）

# Involvement of chemokine receptor CCR4 in lipopolysaccharide-induced depressive-like behavior

Ritsuki Sano, Yuta Hara, Moeka Kitagawa, Tatsuma Honzawa, Kazuhiko Matsuo, Takashi Nakayama

*Div. Chemothr., Kindai Univ. Fac. Pharm.*

Depression is a typical psychiatric disorder with a lifetime prevalence rate of approximately 15%. Although the details of the pathogenesis of depression are still unknown, inflammation is considered as the underlying mechanism leading to depression. The chemokine receptor CCR4 is a major regulator of migration of regulatory T cells (Tregs) and Th17 cells. It has been reported that serum levels of CCR4 ligands are increased in patients with depression. However, the involvement of CCR4 in the pathogenesis of depression is still unknown. Here, we investigated the role of CCR4 in depression using a lipopolysaccharide (LPS)-induced depression mouse model. We found that CCR4-deficient mice displayed a significant increase in immobility time in the forced swim test. Flow cytometry analysis revealed that LPS-induced increase in Treg, but not Th17 cell, migration into the brain was decreased in CCR4-deficient mice. Moreover, CCR4 deficiency increased M1 macrophages were increased, while M2 macrophages were decreased in the brain. Similar to CCR4-deficient mice, treatment with selective CCR4 inhibitor decreased Tregs and M2 macrophages in the brain and increased immobility time in the forced swim test. These results suggest that CCR4 suppresses depressive-like behavior via Treg migration into the brain and M2 macrophage polarization.

2-B-SS07-1 学生セッション（口頭）

# KTtp38, a novel pimozide derivative, suppresses T-type calcium channel-dependent somatic and visceral pain

Tsukasa Hatakeyama[1], Maho Tsubota[1], Yuriko Iba[1], Yoshihito Kasanami[1], Fumiko Sekiguchi[1], Takuya Takuya[2], Naoki Toyooka[2], Atsufumi Kawabata[1]

*[1]Lab. Pharmacol. Pathophysiol., Fac. Pharm., Kindai Univ., [2]Fac. Engineer., Univ. Toyama*

$H_2S$ generated by three different enzymes including cystathionine-$\beta$-synthase (CBS) contributes to somatic and visceral pain by enhancing the activity of $Ca_v3.2$, an isoform of T-type $Ca^{2+}$ channels (T-channels). Most recently, we have developed KTtp38, a novel derivative of the antipsychotic pimozide, that potently inhibits T-channels, but has little affinity to $D_2$ receptors. Here, we investigated the effects of KTtp38 on $Ca_v3.2$-dependent pain, i.e. the somatic and/or colonic pain/hypersensitivity caused by $Na_2S$, an $H_2S$ donor, or butyrate, and also on the colonic hypersensitivity caused by 2,4,6-trinitrobenzene sulfonic acid (TNBS) in mice. Oral administration of KTtp38 potently suppressed somatic and visceral pain following intraplantar and intracolonic (i.col.) administration of $Na_2S$, respectively, and the colonic distention hypersensitivity following repeated i.col. butyrate. A single i.col. TNBS caused delayed colonic distention hypersensitivity accompanied by colonic CBS upregulation, which was inhibited by i.p. aminooxyacetic acid, a CBS inhibitor or deletion of $Ca_v3.2$ gene. Oral or i.p. KTtp38 suppressed the TNBS-induced colonic hypersensitivity. Thus, KTtp38 suppresses $Ca_v3.2$-dependent somatic and visceral pain, and is considered useful to treat pathological pain involving $H_2S$ and/or $Ca_v3.2$.

2-B-SS07-2 学生セッション（口頭）

# Role of HMGB1 and anaphylatoxin C5a in the development of painful peripheral neuropathy in leptin receptor-deficient *db/db* mice and high fat diet-fed type 2 diabetic mice

Kaichi Sakuma[1], Haruka Nakano[1], Shiori Iwane[1], Maho Tsubota[1], Fumiko Sekiguchi[1], Yasuko Tomono[2], Masahiro Nishibori[2], Atsufumi Kawabata[1]

*[1]Lab. Pharmacol. Pathophysiol., Pharm., Kindai Univ., [2]Dept Transl Res Drug Dev, Okayama Univ Grad Sch Med Dent Pharmaceut Sci*

We have demonstrated that high mobility group box 1 (HMGB1) derived from macrophages (Mϕ) and anaphylatoxin C5a participate in chemotherapy-induced peripheral neuropathy, which is prevented by thrombomodulin alfa (TMα) that causes thrombin-dependent HMGB1 degradation and thrombin-activatable fibrinolysis inhibitor (TAFI) activation. Thus, we investigated possible involvement of HMGB1 and C5a degradable by the activated TAFI (TAFIa) in the development of diabetic peripheral neuropathy (DPN) in mice with diabetes mellitus (DM). Repeated administration of TMα or an anti-HMGB1-neutralizing antibody prevented DPN development in type 2 DM (T2DM) models, i.e. leptin receptor-deficient *db/db* mice and high fat diet/low dose streptozotocin (STZ)-induced DM mice, but not in STZ-induced type 1 DM mice. Ethyl pyruvate able to inhibit HMGB1 release from Mϕ, minocycline, a Mϕ/microglia inhibitor, liposomal clodronate, a Mϕ depletor, and DF2593A, a C5a receptor (C5aR) antagonist, reduced or abolished DPN development in the T2DM mice that had upregulation of C5aR, but not HMGB1, in the sciatic nerve. Our data suggest that Mϕ-derived HMGB1 and C5a/C5aR participate in DPN development accompanying T2DM, which can be prevented by TMα that causes thrombin-dependent inactivation of HMGB1 and activation of TAFI followed by C5a degradation.

2-B-SS07-3 学生セッション（口頭）

# Stress sensitivity in a mouse model of low back pain

Yamaji Yasuhito[1], Seiji Kanazawa[1], Sena Washizu[1], Kazunari Mori[1], Oki Hoshino[1], Noriyasu Ota[2], Hiroaki Motohashi[2], Yoshihiko Minegishi[2], Eri Segi(Nishida)[1]

*[1]Dept. of Biol. Sci. and Tech., Tokyo Univ. of Sci,[2]Biological Science Research, Kao Corporation*

This study aims to understand the connection between chronic low back pain, stress, and depression by developing a mouse model. To develop a low back pain model in mice, Complete Freund's Adjuvant (CFA) was administered to the muscle around lumbar region. To evaluate locomotion and balance ability, the balance beam test was performed. Goal-reaching time was increased from day 14 to day 28 in the CFA-treated group, indicating a long-term decline in locomotor performance. To examine the central influence of low back pain model in the hippocampus, we examined the expression of microglia marker Iba1 and neuorogenic marker NeuroD1 in the dentate gryus (DG) of hippocampus 28 days after CFA administration. Iba1-positive cells were increased, and NeuroD1-positive cells were decreased in the DG of the CFA-treated group, indicating long lasting influence of low back inflammation on the hippocampus. Next, we evaluated the influence of the low back pain model on the responses to chronic stress. Mice were exposed to 10 days of social defeat stress before CFA treatment. Although social avoidance behavior was observed shortly after stress, it dissipated 38 days later in the stress alone group. In contrast, social avoidance behavior was more pronounced in CFA-treated mice than in mice exposed to stress alone. These results demonstrate that CFA-induced low back pain model showed long lasting locomotion and balance inability, histological changes in the hippocampus, and exacerbating the stress response. The developed model in this study can aid in elucidating the neuronal mechanism of low back pain and understanding how low back pain intensifies stress.

2-B-SS07-4 学生セッション（口頭）

# FROUNT-mediated pain control of Anti-alcoholism drug disulfiram

Kota Matsuura[1,2], Yuya Terashima[2], Ryoji Fujizuka[1,2], Tasuku Hayashi[1,2], Arisa Ohta[1], Kosei Nagai[1,2], Daisuke Yamada[1], Kosho Makino[3], Hideyo Takahashi[3], Chihiro Nozaki[4], Koji Matsushima[2], Akiyoshi Saitoh[1]

*[1]Lab Pharmacol, Fac Pharm Sci, Tokyo Univ of Science, [2]Research Institute for Biomedical Science, Tokyo Univ of Science, [3]Lab Pharmacol, Fac Lab Medicinal Chemistry Sci, Tokyo Univ of Science, [4]Global Center for Science and Engineering, Major in Bioscience, Waseda University*

Astrocytes are important regulators of CNS functions, including neurotransmission, and play an important role in the etiology of pain. Recently, we have shown that the alcoholism drug disulfiram (DSF) exerts its anticancer effects by directly binding to FROUNT, a protein that promotes signaling through chemokine receptor (CCR)2 and CCR5 expressed on macrophages, and by inhibiting FROUNT function (Nature Communications. 2020: 609). In addition, it has been reported that chemokine receptors including CCR2 and CCR5 are involved in pain regulation. The purpose of this study was to verify the analgesic effects of DSF on various pain models. Formalin model mice were used to evaluate acute nociceptive pain, reserpine model mice were used to evaluate chronic nociceptive pain, and sciatic nerve partial ligation model mice were used to evaluate neuropathic pain. DSF showed analgesic effects on these pain models. In addition, the reduction of pain threshold in reserpine model mice was not observed in FROUNT-deficient mice. And we found that FROUNT-positive cells co-localized with GFAP-positive astrocytes in the dorsal horn of the spinal cord. These results suggest that DSF, an existing treatment for alcohol dependence, may become a new pain treatment with a new mechanism.

# Prophylactic repeated administration of Mirogabalin suppressed Vincristine-induced mechanical allodynia

Ueda Yuki[1], Masahito Sawahata[2], Toshiaki Kume[2], Daisuke Uta[2]

*[1]Department of Applied Pharmacology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, [2]Department of Applied Pharmacology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama*

Chemotherapy-induced peripheral neuropathy (CIPN) is one of the serious side effects of cancer chemotherapy and significantly reduces the quality of life of patients. However, there are no effective treatments or therapeutic agents for CIPN. Therefore, it is a serious problem in the continuation of anticancer drug therapy. A novel gabapentinoid, mirogabalin (MGB), is used as a treatment for neuropathic pain. In this study, in order to develop a new treatment, we investigated the preventive effects of MGB on mechanical allodynia in a mouse model of vincristine (VCT) -induced peripheral neuropathic pain (VIPN). A single oral administration of MGB dose-dependently inhibited VCT-induced mechanical allodynia. Next, to investigate the action sites of MGB, we evaluated the topical analgesic effects on VIPN model mice. We found that intrathecal injection suppressed VCT-induced mechanical allodynia but intradermal injection into the footpad did not. Furthermore, intrathecal injection of MGB in healthy mice did not affect locomotor activity. In addition, prophylactic repeated administration of MGB suppressed VCT-induced mechanical allodynia. These results indicate that MGB prophylactically suppresses VIPN by acting on the spinal dorsal horn. MGB may be an effective therapeutic agent for VIPN.

2-B-SS07-6 学生セッション（口頭）

# Analysis of antipruritic effects of SSRIs using various atopic dermatitis model mice

Kosuke Matsuda, Hikaru Ishisaka, Keita Hori, Masahito Sawahata, Toshiaki Kume, Daisuke Uta

*Dept. Appl. Pharmacol, Grad. Sch. Med. & Pharmaceut. Sci, Univ. Toyama*

Atopic dermatitis (AD) is the most common inflammatory skin disease with chronic itch. The pathophysiology of AD is complex and multifactorial. *The* mechanism of chronic itch is poorly understood. Selective serotonin reuptake inhibitors (SSRIs) are used to treatment of depressants. Recently, it is suggested that SSRIs have potential treatments of AD via antipruritic, antimicrobial and immunomodulatory effects. Here, we investigated whether paroxetine, which is the most potent in SSRIs, exerts an antipruritic effect using NC/Nga (NC) mice (Matsuda et al., 1997) and novel AD model mice (FADS mice; Nunomura et al., 2019). The number of scratching behaviors were increased in these model mice as compared with healthy mice. In addition, we examined the effect of paroxetine (10 mg/kg) by single intraperitoneal injection to NC or FADS mice. Paroxetine suppressed scratching behaviors in these mice while $H_1$ receptor antagonist, terfenadine did not. Furthermore, paroxetine did not affect total distance moved in these mice. These results suggest that SSRIs exert the antipruritic effects for chronic itch by AD.

# Establishment of a novel nerve organoid for sequential analyses of morphological and functional changes underlyingperipheral neuropathy pathogenesis

Ryosuke Ogido[1], Madoka Koyanagi[2], Akari Moriya[1], Mamiko Saigo[3], Satoshi Ihida[4], Tomoko Teranishi[4], Kazuo Matsubara[5], Tomohiro Terada[3], Akira Yamashita[2], Satoshi Imai[2,3]

*[1]Dept. Clin. Pharmacol. Therap., Fac. Pharmaceut. Sci., Kyoto Univ., [2]Dept. Med. Neuropharmacol., Sch. Pharmaceut. Sci., Wakayama Med. Univ., [3]Dept. Clin. Pharmacol. Therap., Kyoto Univ. Hosp., [4]New Business Promo. Div., Panel Semicon Lab., Sharp Corp., [5]Sch. Pharmaceut. Sci., Wakayama Med. Univ.*

Few *in vitro* experimental systems have been optimized for the analysis of the peripheral nervous system (PNS) aimed at elucidating the complex mechanisms underlying the development of peripheral neuropathy. To address this issue, we developed a novel sensory nerve organoid derived from rat embryonic dorsal root ganglion. An innovative advance of present study is that the organoid was composed of independent ganglionic and axonal bundle morphology, which contained unmyelinated C-fibers and A-fibers stereo-myelinated by Schwann cells. After nerve ablation, the axons had almost completely regenerated 2 weeks after injury, indicating that organoids have characteristics specific to the PNS. We also confirmed the mRNA expression of functional molecules (ion channels and receptors), which play important roles in pain transmission, in neuronal cell bodies of organoids. Furthermore, present $Ca^{2+}$ imaging analysis showed that focal application of KCl (30 mM) onto the nerve endings induced $Ca^{2+}$ influx into the neuronal cell bodies. One day after axonal transection, the mRNA expression of stress inducible genes increased in the ganglion. Taken together, this organoid enables real-time evaluation of subtle changes in the PNS, and is considered to be a useful tool for further developing peripheral neuropathy research.

2-B-SS07-8 学生セッション（口頭）

# Involvement of hippocampal microglial activity in persistent pain in a mouse model of knee osteoarthritis

Kenta Yamamoto, Youki Nakamura, Kazue Nakashima, Norimitsu Morioka

*Dept. Pharmacol., Grad. Sch. Biomed. Health Sci., Hiroshima Univ.*

Microglia are involved in induction of chronic pain. In knee osteoarthritis (OA), spinal microglia are activated, but the involvement of brain microglia remains unclear. Therefore, we examined the role of brain microglia in mechanical hypersensitivity in OA.

The OA model was prepared by administration of monoiodoacetate (MIA) into the left knee joint cavity of ddY male mice . Brain slices prepared after MIA administration were stained with an anti-ionized calcium binding molecular 1 (Iba1) antibody, a marker of microglia. Pain thresholds were measured using von Frey filaments. Cartilage damage was evaluated by Safranin O staining.

Activation of microglia in the hippocampus was observed from 4 weeks after MIA administration, peaking at 6 weeks and continuing thereafter. The decreased pain threshold and knee joint damage were observed continuously from 2 weeks after MIA administration. Furthermore, at 5 weeks after MIA administration, local administration of clodronate liposome, which deplete microglia, significantly prevented the decreased pain threshold. These results indicate that there is temporal lag between knee joint damage and pain and activation of hippocampal microglia in the OA model mice, and that these cells are involved in persistent pain.

2-B-SS08-1 学生セッション（口頭）

# Microglial regulation of extracellular ATP released by neuronal activity

Suzuki Hideaki[1,2], Eiji Shigetomi[1,2], Yukiho Hirayama[1], Yukari Takahashi[3], Kazuhiro Ikenaka[4], Kenji Tanaka[5], Fusao Kato[3], Haruhiko Bito[6], Schuichi Koizumi[1,2]

*[1]Dept Neuropharmacol, Interdiscipl Grad Sch Med, Univ Yamanashi, [2]Yamanashi GLIA Center, [3]Dept Neurosci, Jikei Univ, [4]Div Neurobiol and Bioinfo, NIPS, [5]Div Brain Sci, Inst Adv Med Res, Keio Univ Sch Med, [6]Dept Neurochem, Grad Sch Med, Univ Tokyo*

Extracellular ATP (ATPo) is a signaling molecule involved in neurotransmission and neuron-glia signaling and is known to be involved in psychiatric and neurological disorders. However, the exact mechanism of ATP release and the responsible cells under physiological conditions are poorly understood due to the lack of understanding of the spatiotemporal dynamics of ATPo. Using a genetically encoded G protein-coupled receptor activation-based ATPo sensor called $GRAB_{ATP1.0}$, we imaged ATPo near astrocytes in the CA1 region of acute hippocampal slices. Electrical stimulation of the Schaffer collateral resulted in ATPo rise in astrocytes. The ATPo rise was inhibited by TTX, but still remained in the presence of D-APV/CNQX, suggesting that the source of activity-dependent ATP release could be presynaptic sites of neurons. Microglia depletion by PLX5562 prolonged the duration of ATPo rise, suggesting that microglia expressing NTPDase1 rapidly degrade ATPo. Consistently, POM1, a NTPDase inhibitor, increased stimulus-induced ATPo rise. Knockout of $IP_3R2$, a major source of $Ca^{2+}$ in astrocytes, did not alter the ATPo rise, suggesting the minor contribution of astrocytic $Ca^{2+}$ to ATPo. Overall data show that neuronal activity induces ATP release from axons or axon terminals, which is negatively regulated by microglia in physiology.

2-B-SS08-2 学生セッション（口頭）

# Role of microglia in dendritic spine changes of dentate gyrus granule cells following cerebral ischemia.

Okada Momoka[1], Shuma Nakazawa[1], Hitomi Takahashi[2], Natsumi Yamaguchi[1], Jin Nakatani[1], Toshinori Sawano[1], Hidekazu Tanaka[1]

*[1]Lab. Pharmacol., Grad. Sch. Life Sci., Ritsumeikan Univ., [2]Lab. Pharmacol., Dept. Biomed Sci., Col. Life sci., Ritsumeikan Univ.*

Microglia contribute to synaptic pruning by synaptic engulfment, which is dependent on neuronal activity. Our middle cerebral artery occlusion (MCAO) mice show an upregulation of neuronal activity in the hippocampal dentate gyrus (DG). Thus, we investigated the changes in dendritic spines in DG granule cells following cerebral ischemia and microglial contribution to it. We showed that the number of dendritic spines of DG granule cells in MCAO mice was lower than that in sham mice. However, microglial depletion with CSF1R inhibitor (PLX3397) inhibited the MCAO-induced reduction of the dendritic spine. These results suggest that microglia are involved in the decrease in the number of dendritic spines of DG granule cells following cerebral ischemia.

# Proper distribution and proliferation of microglia involves mechanosensitive channel Piezo1

Ayato Yamasaki[1,2], Makoto Tsuda[2], Takahiro Masuda[1]

*[1]Div. Neuroimmunol., Med. Inst. Bioreg., Kyushu Univ.,[2]Dept. Mol. Syst. Pharmacol., Grad. Sch. Pharm. Sci., Kyushu Univ.*

The immune system of the central nervous system (CNS) consists primarily of innate immune cells. As the tissue-resident macrophages, microglia, which are distributed at equal intervals in the CNS parenchyma, play a pivotal role in the maintenance of tissue homeostasis. During the CNS disease, on the other hand, microglia increase in number and exert their proper function. However, the molecular mechanism by which microglia control their cellular density during homeostasis and perturbation remains unknown. In the present study, we investigated the role of Piezo1, a mechanosensitive ion channel, in the regulation of microglia density. In the brains of mice with specific deletion of Piezo1 in myeloid cells including microglia (Piezo1-KO), the density and the morphology of microglia didn't differ from those in wild-type controls. However, after pharmacological depletion of microglia with BLZ945, an inhibitor of colony-stimulating factor 1 receptor, Piezo1-KO microglia showed impaired repopulation capacity with less proliferation rate, leading to lower cell density in the brain. Together, these findings suggest that Piezo1 is involved in regulating proper density of microglia following repopulation.

2-B-SS08-4 学生セッション（口頭）

# Expression and function analysis of immune checkpoint molecule LAG-3 in microglia

Motoki Ohshima, Yuta Morisaki, Hidemi Misawa

*Div. Pharmacol., Fac. Pharmacy, Keio Univ*

Microglia are resident innate immune cells in the central nervous system (CNS) and play important roles in the development of CNS homeostasis. In several CNS disorders, excessive activation and neurotoxicity of microglia are observed, but the mechanisms that regulate their activation are still unclear. Immune checkpoint molecules (e.g., PD-1, LAG-3) are expressed on activated immune cells and regulate their activation in peripheral immunity. We hypothesized that immune checkpoint molecules could also contribute to the regulation of microglial activation.

First, we analyzed the expression of immune checkpoint molecules in activated microglia using BV2, a mouse microglia cell line. We found that BV2 activated by IFN-γ expressed LAG-3 and the STAT1 signaling pathway was involved in the expression of LAG-3. To investigate the function of LAG-3 in activated microglia, we treated IFN-γ-activated BV2 with an antagonistic anti-LAG-3 antibody to inhibit LAG-3 function. The result showed that the inhibition of LAG-3 increased nitric oxide (NO) production upon IFN-γ activation, indicating that LAG-3 could be suppressing microglial activation.

Our results suggest that activated microglia express LAG-3 and that LAG-3 could function as a negative feedback mechanism to regulate microglial activation.

2-B-SS08-5 学生セッション（口頭）

# Neuronal activity-regulated interaction between microglia and myelin

Mikami Koki[1], Yuji Ikegaya[1,2], Ryuta Koyama[1,2]

*[1]Lab Chem Pharmacol, Grad Sch Pharmaceut Sci, Univ Tokyo, [2]Inst AI Beyond, Univ Tokyo*

The proper formation and maintenance of myelin are essential for brain function. It has been found that myelination is regulated in a neuronal activity-dependent manner, resulting from interactions between neurons and oligodendrocytes. Recent reports also suggest that microglia play a crucial role in the formation and maintenance of myelin. However, the specifics of these microglia-myelin interactions remain largely unexplored. To investigate these interactions, we used a mouse cortical slice culture system. This system allowed us to visualize the tripartite relationship between microglia, neurons, and oligodendrocytes using tissue staining and live imaging techniques. This cortical slice culture system was set up by culturing slices from the cortex of 6-day-old mouse brains. Immunostaining of cultured sections with myelin and axon markers revealed that myelin forms on the axons, exhibiting a temporal progression comparable to that seen in mouse cortical sections in vivo. We then examined the impact of neuronal activity on microglia-myelin interactions by modulating neuronal activity using DREADD systems. Our results showed that changes in neuronal activity could have a bidirectional impact on the phagocytosis of myelin by microglia. Our live imaging further disclosed a myelin-like oligodendrocyte structure being phagocytosed by the microglia. In summary, our work has established a cortical slice culture system and suggested that neuronal activity influences the phagocytosis of myelin by microglia.

2-B-SS09-1 学生セッション（口頭）

# Social defeat stress enhances the rewarding effects of cocaine through $\alpha_{1A}$ adrenoceptors in the medial prefrontal cortex of mice

Murata Haruka[1], Atsushi Saito[2], Kazuhei Niitani[2], Jumpei Nagasaki[1], Atsuki Otoda[2], Yusuke Chujo[2], Junko Yanagida[2], Naoya Nishitani[1,2], Satoshi Deyama[1,2], Katsuyuki Kaneda[1,2]

*[1]Lab. Mol. Pharmacol., Sch. Pharmaceut. Sci., Kanazawa Univ., [2]Lab. Mol. Pharmacol., Inst. Med., Pharmaceut., Health Sci., Kanazawa Univ.*

Various stressors potentiate the rewarding effects of cocaine, which contribute to cocaine craving. However, it remains unclear whether psychosocial stress enhances the rewarding effects of cocaine. To address this issue, we employed a cocaine-conditioned place preference (CPP) paradigm combined with social defeat (SD) exposure and investigated the effects of acute SD stress on cocaine reward in mice. We found that SD stress immediately before the posttest significantly increased cocaine CPP, and systemic blockade of $\alpha_1$ adrenoceptors ($\alpha_1$-ARs) suppressed this increase. Fiber photometry recordings with $GRAB_{NE1m}$ sensors revealed increased noradrenaline (NA) levels in the medial prefrontal cortex (mPFC) during SD. Moreover, the SD stress-induced enhancement of CPP was suppressed by intra-mPFC infusion of an $\alpha_1$-AR antagonist. *In vitro* whole-cell recordings showed that silodosin, an $\alpha_{1A}$-, but not $\alpha_{1B}$- or $\alpha_{1D}$-, AR antagonist, inhibited NA-induced depolarizing currents and facilitation of excitatory synaptic transmissions. Consistently, intra-mPFC silodosin infusion suppressed the SD stress-induced CPP enhancement. Additionally, chemogenetic inhibition of mPFC pyramidal cells and intranasal silodosin injection attenuated the CPP enhancement. These findings suggest that NA stimulation of $\alpha_{1A}$-ARs and the subsequent activation of mPFC pyramidal cells may contribute to SD stress-induced amplification of the rewarding effects of cocaine, and intranasal silodosin injection may hold therapeutic potential for stress-associated cocaine craving.

# Role of dopaminergic modulation of glutamatergic transmission from the medial prefrontal cortex to the basolateral amygdala in acute social defeat stress-induced enhancement of cocaine craving

Atsushi Saito[1], Hirohito Esaki[1], Haruka Murata[2], Xiyan Ni[1], Yusuke Chujo[1], Yuki Hirano[1], Yuno Mukai[1], Naoya Nishitani[1,2], Satoshi Deyama[1,2], Katsuyuki Kaneda[1,2]

*[1]Lab. Mol. Pharmacol., Inst. Med., Pharmaceut., Health Sci., Kanazawa Univ., [2]Lab. Mol. Pharmacol., Sch. Pharmaceut. Sci., Kanazawa Univ.*

Stress potentiates cocaine craving by enhancing the rewarding effects of cocaine, yet the mechanisms underlying this process remain unclear. To address this issue, we examined the role of dopaminergic transmission in the medial prefrontal cortex (mPFC) and basolateral amygdala (BLA), and of reciprocal interaction between these nuclei, using a cocaine conditioned place preference (CPP) paradigm combined with acute social defeat (SD) stress in mice. Brief SD exposure before the posttest enhanced cocaine CPP, which was significantly suppressed by systemic, bilateral intra-mPFC, or bilateral intra-BLA injection of SCH23390 (SCH), a dopamine D1 receptor antagonist. Unilateral mPFC and contralateral BLA injections of SCH also suppressed the stress-induced CPP enhancement, suggesting the dopaminergic modulation of reciprocal glutamatergic connection between the mPFC and BLA. Accordingly, simultaneous injections of SCH into the unilateral mPFC and NBQX, an AMPA receptor antagonist, into the contralateral BLA, inhibited the enhanced CPP. By contrast, simultaneous infusions of NBQX to the unilateral mPFC and SCH to the contralateral BLA failed to affect the CPP enhancement. Moreover, selective inhibition of glutamatergic projections from the mPFC to the BLA with the chemogenetic technique suppressed the enhancement of CPP. These findings suggest that dopaminergic inputs to the mPFC and BLA may modulate glutamatergic transmission from the mPFC to BLA, but not from the BLA to mPFC, which contributes to stress-induced potentiation of cocaine craving.

2-B-SS09-3 学生セッション（口頭）

# Chronic social stress causes long-term structural alterations of neuronal projections to the medial prefrontal cortex in mice

Yuki Okuda[1], Ryota Shinohara[1], Hirokazu Sonobe[1], Yuzuki Maruyama[1], Masahiro Yamaguchi[2], Kei Ito[2], Akinori Sato[2], Fumitaka Osakada[2], Tomoyuki Furuyashiki[1]

*[1]Div. of Pharmacol., Grad. Sch. of Medicine, Kobe University, [2]Lab. of Cell. Pharmacol., Dept. of Basic Medical Sci., Grad. Sch. of Pharmaceut. Sci., Nagoya University.*

Chronic social stress affects emotional and cognitive functions and risks psychiatric illnesses such as depression. Rodent studies have shown that chronic social stress induces dendritic atrophy of pyramidal neurons in the medial prefrontal cortex (mPFC), leading to depression-related behaviors. However, how chronic social stress remodels neuronal circuits connected with mPFC neurons is unknown. Here we examined the brain-wide effects of chronic social stress on neuronal projections to the mPFC in mice. Chronic social stress induced behavioral changes, such as social avoidance, decreased reward-directed behavior, and cognitive impairment, which lasted over a month. By unilaterally injecting the retrograde rabies viral vector expressing a fluorescent protein into the mPFC, we visualized the neurons sending axons to the mPFC in more than 150 brain regions. Fluorescently labelled cells in the hippocampus and piriform cortex decreased in stressed mice, while they increased in the orbitofrontal cortex. All these changes were pronounced over a month after the stress. These findings demonstrate that chronic social stress causes long-lasting structural alterations of neuronal projections to the mPFC, which might contribute to post-stress consolidation of depression-related behaviors.

2-B-SS09-4 学生セッション（口頭）

# Metabolic changes in selective brain regions induced by chronic stress in mice and their involvement in emotional disturbance

Ota Kohei[1], Hirotaka Nagai[1], Wenran Qiu[1], Io Horikawa[1], Midori Nagai[1], Chisato Numa[1], Shuichi Shimma[2,3], Tomomi Yamashita[4], Taro Kato[4], Tomoyuki Furuyashiki[1]

*[1]Div Pharmacol, Grad Sch Med, Kobe Univ, [2]Dept Biotechnol, Grad Sch Engineering, Osaka Univ, [3]Omics Innovation Res Lab, Osaka Univ Shimadzu, [4]Pharmacol Res Unit, Sumitomo Pharma*

Chronic stress causes depressed mood and cognitive deficits and predisposes to mental illness. Brain metabolic changes are linked to stress pathology, but their molecular mechanism and functional significance remain unknown. In this study, we exposed C57BL/6N male mice to chronic social defeat stress and quantified the metabolites of central metabolic pathways by mass spectrometry imaging. We examined multiple stress-associated brain regions in susceptible mice, which showed stress-induced social avoidance, and resilient mice, which did not. In the medial prefrontal cortex and the hippocampus, chronic stress increased glycolytic metabolites of susceptible mice but not resilient mice. Among susceptible mice, we found a positive correlation between the glycolytic metabolite levels in the medial prefrontal cortex and social avoidance. Knockdown of glucose transporter in this region ameliorated stress-induced depressive-like behavior. In addition, knockdown of the glucose transporter specifically in the hippocampal neurons projecting to the medial prefrontal cortex suppressed stress-induced cognitive dysfunction. These findings suggest that chronic stress induces diverse metabolic alterations across multiple brain regions, with central metabolic changes in the prefrontal cortex and the hippocampus playing a crucial role in stress-related pathology associated with mental illness.

2-B-SS09-5 学生セッション（口頭）

# The sucrose intake changes stress-induced behavior via dysfunction of noradrenergic nervous system.

Takatoshi Sakata[1], Akihiro Mouri[1,4], Kazuo Kunisawa[1], Masaya Hasegawa[1], Takaya Nishikawa[1], Masao Takemura[2,5], Hidetoshi Matunami[5], Kuniaki Saito[2,3,4], Toshitaka Nabeshima[3,4]

*[1]Dept. Regulatory Sci., Grad. Sch. Health Sci., Fujita Health Univ., [2]Dept. Disease Control & Prevention., Grad. Sch. Health Sci., Fujita Health Univ., [3]Lab. Health & Medical Sci. Innov., Grad. Sch. Health Sci., Fujita Health Univ., [4]NPO. J-DO, [5]RECHS*

Lifestyle habits have attracted attention as environmental factors of depression.
We analyzed lifestyle habits in high-risk subjects of major depressive disorder (HRMDD). In our analysis of lifestyle habits in HRMDD, we observed elevated sucrose intake.
To investigate how sucrose intake affects stress-induced depression-like behaviors, mice took sucrose liquid freely were subjected to chronic unpredictable mild stress (CUMS). The sucrose intake attenuated CUMS-induced hyperactivity and aggressive behavior but not social deficit. Unexpectedly, the sucrose intake under CUMS impaired recognition memory.
CUMS reduced noradrenaline (NA) tissue levels in the prefrontal cortex. Sucrose intake under CUMS conditions mitigated reduction in NA levels, although it slowed down the turnover of NA, which associated with a decrease in the expression of adrenergic $\alpha 1$ receptors and an increase in the expression of adrenergic $\alpha 2$ receptors.
In this study, it is suggested that increased sucrose intake in HRMDD serves to attenuate stress-induced aggression and hyperactivity. However, this comes with the unintended consequence of impairing cognitive function.These contradictory findings may be attributed to changes observed in NA tissue levels and receptor expression in prefrontal cortex.

# The role of 5-HT neurotransmission in the regulation of the motivation for wheel running in mice

Kazuhei Niitani[1], Ryoma Nishida[2], Naoya Nishitani[1,2], Satoshi Deyama[1,2], Katsuyuki Kaneda[1,2]

*[1]Lab. Mol. Pharmacol., Inst. Med., Pharmaceut., Health Sci., Kanazawa Univ., [2]Lab. Mol. Pharmacol., Sch. Pharmaceut. Sci., Kanazawa Univ*

Behavioral addiction, defined as an uncontrollable desire to repeat a certain behavior despite negative consequences, has become a social problem. Here, we investigated the role of 5-HT neurotransmission in the nucleus accumbens (NAc) in motivation for wheel running as a model of behavioral addiction in male C57BL/6J mice (> 6 weeks old). Systemic administration of a 5-$HT_{1A}$ antagonist (WAY100635) increased wheel rotations, while a 5-$HT_{2A}$ or 5-$HT_{2C}$ antagonist (volinanserine or SB242084, respectively) decreased them. In the open field test (OFT), WAY100635 or volinanserine did not affect locomotion, but SB242084 increased it. Intra-NAc infusion of SB242084 reduced wheel rotations without altering locomotion in the OFT, whereas intra-NAc infusion of WAY100635 or volinanserine did not affect wheel rotations. Immunohistochemical analysis revealed that wheel running increased the number of c-Fos-positive cells in the NAc, and this increase was reduced by systemic administration of SB242084. Additionally, whole-cell recordings revealed that bath application of a 5-$HT_{2C}$ receptor agonist (lorcaserin) increased the number of evoked action potentials and spontaneous excitatory postsynaptic currents in NAc neurons. Our results suggest that the activation of the NAc via 5-$HT_{2C}$ receptor stimulation regulates the motivation for wheel running.

2-B-SS09-7 学生セッション（口頭）

# Role of the transcription factor CREB in antidepressant effects in the hippocampus

Yui Sayoko, Yutaka Kanda, Mio Kutsuzawa, Sayoko Suzuki, Eri Segi-Nishida

*Dept. of Biol. Sci. and Tech., Tokyo Univ of Sci*

Among antidepressant treatments, electroconvulsive therapy (ECT) is the most efficacious treatment for depression, but the cellular mechanisms underlying the actions of ECT are unknown. Electroconvulsive stimulation (ECS), an animal model of ECT, robustly stimulates hippocampal neurons and gene transcription and enhances adult neurogenesis in the dentate gyrus (DG) of hippocampus. In this study, we focused on CREB, a transcription factor expressed in the hippocampal DG and activated by signals in response to extracellular stimuli. To clarify the role of CREB on increased neurogenesis and expression change in the DG by chronic ECS, we generated adeno associated virus (AAV) expressing GFP and artificial microRNA targeting CREB (miR-CREB) and injected it into the mouse DG. Knockdown (KD) of CREB expression in the DG was confirmed by immunostaining 5 weeks after AAV injection. Eleven times of ECS were administered to CREB KD animals and immunostaining was performed. The repeated ECS significantly increased the number of NeuroD1-positive neural progenitor cells and doubulecortin-positive immature neurons and their dendric elongation in control mice. We found that these cellular changes by repeated ECS were attenuated in the DG of CREB KD animals. We will continue to elucidate whether CREB is involved in these ECS-induced changes in gene expression and whether they correspond to histological changes.

2-B-SS09-8 学生セッション（口頭）

# Identification of the medial prefrontal circuit responsive to the atypical antipsychotic drug clozapine.

Yumi Hirato[1], Kaoru Seiriki[1], Shohei Yamada[1], Leo Kojima[1], Atsushi Kasai[1,2], Takanobu Nakazawa[3], Hitoshi Hashimoto[1,4,5,6,7]

*[1]Lab. Mol. Neuropharmacol., Grad. Sch. Pharmaceut. Sci., Osaka Univ., Osaka, Japan, [2]Drug Innov. Cent., Grad. Sch. Pharmaceut. Sci., Osaka Univ., Osaka, Japan, [3]Dept. Biosci., Fac. Life Sci., Tokyo Univ. Agr., Tokyo, Japan, [4]Mol. Res. Ctr. Child. Ment. Dev., United Grad. Sch. Child Dev., Osaka Univ., Osaka, Japan, [5]Div. Biosci., Inst. Datability Sci., Osaka Univ., Osaka, Japan, [6]Transdimensional. Life Imaging Div., Inst. for Open Transdisciplinary Res. Initiatives, Osaka Univ., Osaka, Japan, [7]Dept. Mol. Pharmaceut. Sci., Grad. Sch. of Med., Osaka Univ., Osaka, Japan*

Clozapine (CLZ) is an atypical antipsychotic drug used for treatment-resistant schizophrenia. The target receptors for CLZ, including dopamine D4 and serotonin 5-HT2A receptors, are expressed in various types of neurons with different expression levels. Although cellular and molecular responses by CLZ have been characterized by various studies, neuronal circuit mechanisms underlying the effect of CLZ remain elusive. Thus, it is important to identify characteristics of neurons functionally responsible for the effect of CLZ. For this purpose, we investigated anatomical features of CLZ-responsive neurons in the medial prefrontal cortex (mPFC) by genetic labeling of CLZ-activated neurons and their axons. We found that CLZ-activated neurons in the mPFC have more projections to the mediodorsal thalamus (MD) and the ventromedial thalamus than the basolateral amygdala, whereas vehicle-activated neurons in the mPFC have similar extent of projections to these three regions. We also found that the MD-projecting neurons in the mPFC were activated by CLZ not only in wild-type mice but in a mouse model of schizophrenia by retrograde tracing and immunohistochemistry for c-Fos. These results suggest that the activation of the mPFC-MD circuit is involved, at least partly, in the mechanism for the therapeutic action of CLZ.

# Development of AAV vectors with optimized cell-type specificity and labeling efficiency using cell-type-specific promoters.

Leo Kojima[1], Kaoru Seiriki[1], Hiroki Rokujo[1], Atsushi Kasai[1], Takanobu Nakazawa[2], Hitoshi Hashimoto[1,3,4,5,6]

*[1]Lab. Mol. Neuropharmacol., Grad. Sch. Pharmaceut. Sci., Osaka Univ., Osaka, Japan,[2]Dept. Biosci., Fac. Life Sci., Tokyo Univ. Agr., Tokyo,[3]Mol. Res. Ctr. Child. Ment. Dev., United Grad. Sch. Child Dev., Osaka Univ., Osaka, Japan, [4]Div. Biosci., Inst. Datability Sci., Osaka Univ., Osaka, Japan,[5]Transdimensional. Life Imaging Div., Inst. for Open Transdisciplinary Res. Initiatives, Osaka Univ., Osaka, Japan,[6]Dept.Mol. Pharmaceut. Sci., Grad. Sch. of Med., Osaka Univ., Osaka, Japan*

Adeno-associated virus (AAV) vectors are powerful tools for cell-type-selective gene delivery to the central nervous system and are promising viral vectors for gene therapy. Despite research on the identification and modification of minimal enhancers and promoters for cell-type-specific gene expression, the application of these enhancers/promoters to AAV often has a trade-off between cell-type specificity and labeling efficiency. Here, we attempted to label targeted neuronal populations specifically and efficiently by optimizing genomic components of AAV. As a result, we established an optimized AAV expression cassette for cell-type-selective and robust gene expression in oxytocinergic, serotonergic and dopaminergic neurons. we also achieved application of oxytocinergic-neuron-selective AAV to labeling the axonal projection patterns on a whole-brain scale in wild-type mice. Our results suggest that the optimization of gene cassettes in AAV vectors can be useful for specific and efficient gene expression toward targeted cell types. These strategies may improve the performance of previously established approaches using AAV vectors with cell-type-specific promoters.

2-B-SS10-2 学生セッション（口頭）

# Axo-axonic cells regulate associative learning.

Nakashima Miki[1], Yuji Ikegaya[1,2], Shota Morikawa[1,3]

*[1]Lab Chem Pharmacol, Grad Sch Pharmaceut Sci, Univ Tokyo, [2]Inst AI Beyond, Univ Tokyo, [3]Lab Mol Neurophysiol, Grad Sch Sci, Univ Tokyo*

The activity and plasticity of excitatory neurons are regulated in a specific way by local inhibitory neurons. Axo-axonic cells (AACs) are a unique type of inhibitory neurons that primarily form synapses onto the axon initial segment of pyramidal neurons. While their anatomical features have been identified, the functional roles of AACs remain unclear. In this study, we used a specific labeling technique to label AACs in the basolateral amygdala (BLA) and found that they have a crucial role in fear conditioning. By using in vivo calcium imaging of AACs in the BLA, we demonstrated their activation in response to salient stimuli, such as foot shock and reward. Moreover, when AACs were inactivated, the activity of pyramidal neurons increased and fear conditioning was impaired. We discovered that the strength of the inhibitory input from AACs differs between active and non-active neurons during fear conditioning. Additionally, we observed that AACs preferentially receive long-range inputs from the basal forebrain and medial geniculate nucleus. These findings suggest that AACs play a central role in representing salient stimuli and are crucial for regulating BLA activity during memory acquisition.

2-B-SS10-3 学生セッション（口頭）

# Membrane potentials of retrosplenial late spiking neurons are not locked with neocortical slow waves

Hiroyuki Mizuno[1], Yuji Ikegaya[1,2]

*[1]Lab. Chem. Pharmacol., Grad. Sch. Pharmaceut. Sci., Univ. Tokyo,[2]Inst. AI Beyond, Univ. Tokyo*

Memory consolidation depends on the interaction between the hippocampus and the neocortex during slow-wave sleep, and the retrosplenial cortex (RSC) is thought to mediate this interaction. The coupling of hippocampal activity with neocortical slow waves plays a crucial role in memory consolidation, but the dynamics of slow waves in the RSC remain unclear. To investigate whether individual neurons of the RSC exhibit synchronized activity with slow waves, we conducted in vivo whole-cell patch clamp recordings to monitor the membrane potentials of RSC neurons simultaneously with local field potential recordings of slow waves from urethane-anesthetized mice. Among the 40 neurons recorded, 21 exhibited membrane potential dynamics synchronized with slow waves, while 19 exhibited brief and frequent depolarizations without phase locking to neocortical slow waves. Analysis of intrinsic membrane properties revealed that the former were regular spiking neurons, whereas the latter were late spiking neurons. These results suggest that regular spiking neurons, but not late spiking neurons, primarily receive inputs from the neocortex, implying parallel processing by these two cell types in the RSC.

2-B-SS10-4 学生セッション（口頭）

# Mechanism of mitochondrial translation inhibition by C9ORF72 dipeptide repeat proteins.

Rio Yamazaki, Kohsuke Kanekura

*Department of Pharmacology, Tokyo Medical University*

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease for which no curative treatment is established. Recently, it has been proposed that insufficient energy production due to mitochondrial dysfunction may underlie the ALS pathogenesis, but the precise mechanism remains to be elucidated. Mutant C9ORF72, the most common ALS-causative gene, produces five dipeptide repeat proteins from the abnormally elongated (GGGGCC) sequence. Among them, poly(GR) has been shown to affect mitochondrial function and impair energy production via unknown mechanisms. We focused on the fact that mitochondria have a translation mechanism similar to that of prokaryotes. The newly synthesized mitochondrial proteins can be visualized using the click chemistry technique under cycloheximide treatment because mitochondrial translation is resistant to cycloheximide. We found that poly (GR) strongly inhibited mitochondrial translation, whereas poly(PR), another Arg-rich C9ORF72-dipeptide, did not. We also found that poly(GR) activated mitochondrial UPR (UPRmt) signaling, especially the ATF4/CHOP axis. Therefore, we speculated that aberrant activation of UPRmt by poly(GR) suppresses mitochondrial translation, leading to insufficient mitochondrial energy production and motor neuronal cell death.

2-B-SS10-5 学生セッション（口頭）

# Modulation of astrocyte activation via the NF-κB pathway by sphingomyelin.

Ryo Kadowaki, Takuya Honda, Hiroyuki Nakamura

*Lab. Chemical Pharmacol., Grad. Sch. Pharmaceut. Sci., Chiba Univ.*

Background: Astrocytes constitute about 20~40% of glial cells, making up the central nervous system. Astrocytes usually maintain homeostasis in the extracellular environment, changing their morphology to the activated state in neurodegenerative diseases and releasing inflammatory cytokines. Sphingolipids, one of the lipids, have been reported as a molecule associated with astrocyte activation. Here, we evaluated astrocyte activity by changing sphingomyelin (SM) levels using HASTR/ci35, a conditionally immortalized human astrocyte.

Results & Discussion: Increasing endogenous SM levels by inhibiting neutral sphingomyelinase and the external addition of SM increased protein and mRNA expression of astrocyte activation markers by IL-1α/TNF-α treatment. On the other hand, decreasing intercellular SM by the knockdown of sphingomyelin synthase and ceramide transport protein (CERT) reduced protein and mRNA expression of astrocyte activation markers by IL-1α/TNF-α treatment. We analyzed the NF-κB pathway to elucidate how reducing intracellular SM levels suppresses astrocyte activation. We found that CERT knockdown did not alter phospho-IκB, phospho-p65 expression, and p65 nuclear translocation. Interestingly, protein expression of HDAC3, which negatively regulates the NF-κB pathway, was significantly increased. These results suggest that reducing intracellular SM levels suppress astrocyte activation by inhibiting the NF-κB pathway through the induction of HDAC3 expression.

2-B-SS11-1 学生セッション（口頭）

# 15-hydroxyeicosatrienoic acid can be a novel exacerbating lipid mediator for nasal congestion which stimulates $PGD_2$ and $PGI_2$ receptors.

Noriko Ozaki[1], Naoaki Sakamoto[1], Daiki Horikami[1], Yuri Tachibana[1], Nanae Nagata[1], Koji Kobayashi[2], Yoshino Arai[3], Masayoshi Sone[3], Kazuhiro Hirayama[4], Takahisa Murata[1,2,5]

*[1]Dept. Animal Radiolody., Grad. Agr. & Life Sciences., Univ. of Tokyo,[2]Crs. Food and Animal Systemics., Grad. Agr. & Life Sciences., Univ. of Tokyo,[3]Sone clinic, [4]Dept. Veterinary Public Health., Grad. Agr. & Life Sciences., Univ. of Tokyo,[5]Dept. Veterinary Pharmacol., Grad. Agr. & Life Sciences., Univ. of Tokyo*

Lipid mediators such as prostaglandins and leukotrienes exacerbated nasal congestion in allergic rhinitis (AR) by increasing blood flow and vascular permeability in nasal mucosa. We here aimed to investigate the effect of a lipoxygenase-metabolite of dihomogammalinolenic acid, 15-hydroxyeicosatrienoic acid (15-HETrE) on functional changes of vasculature, since the previous study showed high level of 15-HETrE was detected in the nasal lavage fluid of AR mouse models. Intranasal administration of 15-HETrE caused abdominal breathing, decreased nasal cavity volume, and increased extravasation of dye injected intravenously in mice. Whole-mount immunostaining revealed that 15-HETrE administration relaxed vessels in nasal mucosa. In ex vivo experiments, the treatment of 15-HETrE relaxed mouse aorta pre-contracted by U46619 in a dose-dependent manner. This 15-HETrE-induced relaxation was inhibited by pre-treatment of prostaglandin $D_2$ receptor (DP) or prostacyclin receptor (IP) antagonists. Accordingly, the treatment of 15-HETrE on aorta tended to increase the level of intracellular cAMP. Finally, we showed 15-HETrE was detected in patients who complains of AR-related symptoms. These results indicate 15-HETrE can be a novel exacerbating lipid mediator for nasal congestion which stimulates major prostaglandin receptors DP and IP.

# Involvement of the $Na^+/Ca^{2+}$ exchanger in the automaticity of the pulmonary vein myocardium, but not in the sinoatrial node, as revealed by intracellular ion environment measurement

Ryosuke Odaka, Shogo Hamaguchi, Iyuki Namekata, Hikaru Tanaka

*Dept. Pharmacol., Toho Univ. Fclt. Pharmacent. Sci*

The mechanism for myocardial automaticity may differ among different regions of the heart. In this study, we performed fluorescent ion measurements in cardiomyocytes from the sinoatrial node (SAN), the orthotopic pacemaker, and the pulmonary vein (PV), a potential ectopic pacemaker which may cause atrial fibrillation, focusing on the role of the $Na^+/Ca^{2+}$ exchanger (NCX). Isolated cardiomyocytes from the guinea pig PV and SAN showing automaticity were loaded with the $Ca^{2+}$ indicators Fluo-4 or Indo-1 for high-speed $Ca^{2+}$ imaging. Inhibition of NCX either by SEA0400 or by low $Na^+$ solution decreased the $Ca^{2+}$ transient frequency in PV, but not in SAN. The basal cytoplasmic $Ca^{2+}$ concentration, as well as the number of $Ca^{2+}$ sparks between $Ca^{2+}$ transients, were slightly higher in PV than in SAN. Intracellular $Na^+$ concentration, measured by a $Na^+$ indicator SBFI, was not different between PV and SAN. The equilibrium potential of NCX ($E_{NCX}$) was estimated to be less negative in PV than in SAN. In conclusion, NCX is involved in spontaneous activity in PV, but not in SAN. This is probably because the less negative $E_{NCX}$ and the more negative voltage range for diastolic depolarization in the PV cause a larger driving force for NCX. In the SAN, whose diastolic depolarization largely overlaps with the $E_{NCX}$, the role of NCX in automaticity is limited.

2-B-SS11-3 学生セッション（口頭）

# Epigenetic Regulation in Diabetic Cardiomyopathy

Shunji Hirose[1], Masafumi Funamoto[2], Amiho Muramatsu[1], Miyako Ueno[1], Masaki Imanishi[1], Yasumasa Ikeda[2], Koichiro Tsuchiya[1]

*[1]Dept. of Med. Phamacol. Fac. of Farm. Tokushima Univ.,[2]Dept. of Pharmacol. Tokushima Univ. Grad. Sch. of Biomed. Sci.*

Background: Diabetic cardiomyopathy (DCM) is a complication of diabetes that results in pathological cardiac hypertrophy and fibrosis, leading to cardiac dysfunction. DCM also induces cardiac senescence. Epigenetic changes are involved in cellular senescence. However, there is no suitable DCM model that can be created in a short period of time. The purpose of this study is to establish a mouse model of DCM and elucidate the cardiac epigenetic change using the animals.

Methods and Results: Cardiac myoblast H9c2 cells were treated with AGEs (100 or 200 $\mu$g/mL). AGEs increased the protein levels of p53 and $\gamma$-H2AX as well as the gene expression levels of p21 and p53. Next, we examined a mouse model of type 2 diabetes. C57BL6/N mice (8-week-old, male) were fed a high-fat diet (HFD) and L-NAME (1 g/L) for 4 weeks, followed by daily injections of streptozotocin (STZ) (50 mg/kg/day) for 5 days and sacrificed after another 4 weeks. Picrosirius red staining showed that cardiac fibrosis was increased in L-NAME+HFD+STZ (LHS) mice. LHS mice showed increased gene expression levels of inflammatory cytokines, fibrosis-related genes, and senescence markers. Acetylation and crotonylation levels of histone H3K9 were enhanced in LHS mice.

Conclusion: We established a novel mouse model of DCM, and DCM might be regulated by epigenetic mechanisms.

2-B-SS11-4 学生セッション（口頭）

# Analysis of the effect on cells by the expression of the kidney-specific ubiquitin ligase RNF183, involved in degradation of ion transporters, under hyperosmotic stress.

Yuki Higashi, Takumi Okamoto, Masayuki Kaneko

*Dept. Pharmacol. Therap. Innov., Nagasaki Univ. Grad. Sch. Biomed. Sci*

We have elucidated that the ubiquitin ligase RNF183 is specifically expressed in the kidney, especially in the collecting ducts, which are constantly exposed to hyperosmotic stress. We also identified NKCC1 as a substrate protein of RNF183 using the proximal biotin labeling method with RNF183 fused with the biotin ligase BirA.

In this study, we examined the role of ubiquitination of NKCC1 by RNF183. This analysis was conducted using HEK293 cells expressing RNF183 by the Tet-on system. Our results suggest that RNF183 ubiquitinates NKCC1, promoting its lysosomal degradation. Additionally, in mIMCD cells, which show induced expression of endogenous Rnf183 under hyperosmotic stress, we generated Rnf183-KO cells and examined whether cell death increased or decreased under this stress. The result indicated that the expression of cleaved caspase-3 was elevated in Rnf183-KO cells.

A recent study reported that the expression of RNF183 is upregulated in the colons of patients with inflammatory bowel disease (IBD). Since the molecular mechanisms underlying the development and pathophysiology of IBD are not fully understood, IBD is designated as an intractable disease. Therefore, we plan to analyze the relationship between RNF183 expression and hyperosmotic stress in colon cells and the effects of increased RNF183 expression.

2-B-SS11-5 学生セッション（口頭）

# Glucagon-like Peptide 1 Receptor Agonist Attenuates Diabetic Podocyte Injury

Yoshida Akane, Miyuki Kobara, Hiroe Toba, Tetsuo Nakata

*Department of Clinical Pharmacology, Kyoto Pharmaceutical University*

[Background] Podocytes form the essential components of the glomerular filtration barrier and have a critical role in diabetic kidney disease (DKD). Currently, mounting evidence suggests that glucagon-like peptide 1 receptor agonists (GLP-1RAs), anti-diabetic drugs, have beneficial effects on DKD. However, direct effects of GLP-1RAs on diabetic podocyte injury remain unknown. We investigated whether exendin-4, a GLP-1RA, attenuates hyperglycemia-induced podocytes injury using cultured podocytes and DKD in rat models of type 1 diabetes, and if so mechanism of its beneficial effects. [Methods and Results] Cultured podocytes were exposed to media containing normal (NG; 5 mmol/L) or high glucose (HG; 25 mmol/L) for one week in the presence or absence of exendin-4 (10 nmol/L). HG increased podocytes apoptosis and reduced mRNA expression of novel podocyte markers, synaptopodin and Wilms tumor 1 (WT1). Exendin-4 reduced podocytes apoptosis and restored these mRNA expression, however, these protective effects were attenuated by the co-treatment with wortmannin, a PI3 kinase inhibitor. Exendin-4 also preserved Bcl2 and reduced Bax, protein expression. In *in vivo* study using rat models of streptozotocin-induced type 1 diabetes, exendin-4 suppressed impaired plasma creatinine levels, mesangial expansion, and preserved WT-1-positive podocytes without any changes of plasma glucose levels. [Conclusion] Exendin-4 attenuates hyperglycemia-induced podocyte injury through PI3 kinase signaling pathway, leading to improvement of DKD.

2-B-SS12-1 学生セッション（口頭）

# Oral application of persimmon tannin significantly inhibited the halitosis and pro-inflammatory response in a Porphyromonas gulae dependent periodontal disease in dogs.

Toyooka Megu[1], Mao Kaneki[1], Chiharu Ohira[1], Jumpei Uchiyama[2], Tomoki Fukuyama[1]

*[1]Lab. of Pharmacol., Sch. of Vet. Med., Azabu Univ.,[2]Dept. of Bacteriol., Grad. Sch. of Med. Dent. & Pharmaceut. Sci., Okayama Univ.*

Periodontal disease is a serious problem in the veterinary field, as it is reported that more than 80% of dogs over 6 years of age suffer from periodontal disease. Severe periodontal infection is irreversible; therefore, once the supporting tissues are damaged, there is no possibility of recovery. Therefore, preventive dentistry, such as daily tooth-cleaning and dental gel from early life, at the veterinary hospital as well as at home, is quite important. In this study, we focused on persimmon tannin, a polyphenol extracted from persimmon, and examined the bactericidal, anti-halitosis and anti-inflammatory effects *in vitro* using *Porphyromonas gulae* (*P. gulae*), which is a major contributor to the progression of periodontal disease in dogs. Clinical study in 20 dogs with severe periodontal disease was also conducted by daily oral application of 1% persimmon tannin gel. Whereas persimmon tannins did not alter the growth of *P. gulae*, significant inhibition of $CH_3SH$ production by *P. gulae,* and significant inhibition of IL-6, IL-1$\beta$ and TNF$\alpha$ production by mouse macrophage cell line infected with *P. gulae* were observed in persimmon tannin treated group. *In vitro* anti-halitosis, and anti-inflammatory effects of persimmon tannin were confirmed by clinical experiments in dogs with *P. gulae*-associated periodontal diseases, and one-month oral treatment with 1% persimmon tannin contained dental gel significantly reduced halitosis and *P. gulae* activity. Our findings suggest that oral treatment with persimmon tannin can be a preventive option for periodontal disease in dogs.

2-B-SS12-2 学生セッション（口頭）

# Role of Kv1.6 channel in chondrocytes in osteoarthritis

Tomo Kurata[1], Yoshiaki Suzuki[1], Shinya Tateno[1], Shigeru Miyaki[2], Bernotiene Elva[3], Giles Wayne[4], Hisao Yamamura[1]

*[1]Dept. Mol. & Cell. Pharmacol., Grad. Sch. Pharmaceut. Sci., Nagoya City Univ., [2]Dept. Orthopaedic Surg., Grad. Sch. Biomed. & Health Sci., Hiroshima Univ., [3]Dept. Regenerative Med., Innov. Med. Ctr., [4]Dept. Physiol. & Pharmacol., Cumming Sch. Med., Calgary Univ.*

Osteoarthritis (OA) is a chronic inflammatory disease characterized by a decrease in cartilage matrix, disorders of joint movement, and severe pain. Previous studies have suggested that an increase in intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) in chondrocytes is associated with OA progression. However, the mechanism underlying this increased $[Ca^{2+}]_i$ is unknown. In the present study, we aimed to elucidate this mechanism and its roles in OA progression. Primary chondrocytes were isolated from C57BL/6 mice, and treated with interleukin (IL)-1$\beta$, a major cytokine secreted into synovial fluid during OA. In IL-1$\beta$-treated chondrocytes, resting membrane potential was depolarized, and resting $[Ca^{2+}]_i$ was increased due to the downregulation of voltage-gated $K^+$ channel, Kv1.6. This downregulation of Kv1.6 was also detected in chondrocytes from OA model mice and OA patients. IL-1$\beta$ induced depolarization of mitochondrial membrane potential ($\Delta\Psi m$) and cell death. In contrast, overexpression of Kv1.6 in chondrocytes using adenovirus reduced resting $[Ca^{2+}]_i$, increased $\Delta\Psi m$, and inhibited cell death. In summary, IL-1$\beta$ downregulates Kv1.6 and increases resting $[Ca^{2+}]_i$, resulting in mitochondrial $Ca^{2+}$ overload and subsequent cell death. Our findings may contribute to the understanding of OA pathogenesis and the development of new treatments for OA.

2-B-SS12-3 学生セッション（口頭）

# Intravital imaging to visualize mechanosensing by osteocytes using ATP dynamics

Takegami Hina[1], Takanobu Fukunaga[2], Masamichi Yamamoto[3], Keizo Nishikawa[1]

*[1]Dept. of Med. Life Sys., Grad. Sch. of Biomed., Univ. of Doshisha, [2]Fac. of Eng., Univ. of Kyushu, [3]NCVC*

Osteocytes play a crucial role in regulating bone metabolism through their interaction with osteoclasts and osteoblasts. While osteocytes are believed to act as bone mechanosensors, this hypothesis has only been confirmed in vitro and remains unexplored at the in vivo level. In this study, we developed a novel method to visualize osteocyte responses to mechanical loading in live mice using a two-photon excitation microscope.

To facilitate our research, we developed specialized fixtures using an optical 3D printer and securely anchored bone tissue using biocompatible cement. We selected a strain of mice expressing GO-ATeam2, a fluorescence resonance energy transfer (FRET)-based biosensor for ATP, either in the cytosol or mitochondria, as ATP dynamics are recognized as indicative of osteocyte mechanosensing. We obtained fluorescent images from distinct organelles within the osteocytes. Subsequently, we applied static loads along the tibia's long axis using an actuator and monitored the applied force with a load cell. Fluorescent images were captured under various loading conditions. Our investigation into cytosolic ATP dynamics in osteocytes under bone loading, as analyzed through cytosol-localized GO-ATeam2, revealed no significant differences in ratio values at 1N and 3N compared to 0N. However, a noteworthy decrease was observed at 5N and 7N. Similarly, our examination of intramitochondrial ATP dynamics using mitochondria-localized GO-ATeam2 displayed no significant deviations in ratio values across loading conditions ranging from 1N to 7N when compared to 0N.

Our findings suggest that osteocytes do not respond to low loads below 3N. In contrast, excessive loading above 5N results in alterations in the cellular state of osteocytes. Furthermore, our study implies that osteocytes subjected to 5N and 7N mechanical loading exhibit distinct ATP dynamics within osteocyte organelles as a response to mechanical stimuli at the in vivo level.

# Mechanisms of the inhibition of cancer cell proliferation by LAT1 inhibitors revealed by the changes in the intracellular concentrations and function of individual amino acids

Kou Nishikubo[1], Ryuichi Ohgaki[1,2], Hiroki Okanishi[1], Minhui Xu[1], Hitoshi Endou[3], Yoshikatsu Kanai[1,2]

*[1]Dept. Bio-system Pharmacol., Grad. Sch. Med., Osaka Univ., [2]Integ. Front. Res. Med. Sci. Div., OTRI, Osaka Univ., [3]J-Pharma Co., Ltd.*

Nanvuranlat (JPH203, KYT-0353), an inhibitor for L-type amino acid transporter 1 (LAT1; SLC7A5), suppresses the cancer cell proliferation and tumor growth by inhibiting the uptake of large neutral amino acids into cancer cells. Most previous studies have focused on the inhibition of leucine uptake to describe the pharmacological effects of nanvuranlat, mainly because leucine is an essential amino acid that functions as activating signaling molecules of cellular metabolism. In this study, to elucidate the anti-cancer effects of LAT1 inhibitors in more detail, we focused on changes in the intracellular concentrations of all the LAT1 substrates and their importance on cell proliferation. Surprisingly, high-performance liquid chromatography analysis revealed that only three large neutral amino acids were continuously decreased by the treatment with nanvuranlat. Similar changes were commonly observed in multiple cancer cell lines. Culturing the cells in media depleted with each or all of the three amino acids reduced the intracellular amount of corresponding amino acids, partially recapitulating the effects of nanvuranlat on cell proliferation, amino acid signaling, and cell cycle arrest. These findings contribute to understanding the molecular basis underlying the anti-cancer effects of LAT1 inhibitors.

2-B-SS12-5 学生セッション（口頭）

# Effect of sensory neuron-derived growth factors on acquired resistance to molecular-targeted therapies for epidermal growth factor receptor-mutated non-small cell lung cancer

Hitoshi Makabe[1,2], Michiko Narita[2], Yukari Suda[1,2], Yukino Ide[1], Shin Iizuka[1,2], Yasuyuki Nagumo[2], Naoko Kuzumaki[1,2], Minoru Narita[1,2]

*[1]Dept. Pharmacol., Hoshi Univ., Tokyo, Japan,[2]Div. Pathophysiol., Natl. Cancer Ctr. Res. Inst., Tokyo, Japan*

Not published

2-B-SS12-6 学生セッション（口頭）

# Role of DNA damage response protein BRAT1 in the mechanisms of cell death induced by a novel anticancer compound ACAGT-007a

Tanaka Tatsuya, Sugiura Reiko

*Dept. Molecular Pharmacogenomics. Kindai Univ.*

We have previously identified an anti-cancer compound ACA-28 and its lead compound ACAGT-007a (GT-7) as novel regulators of ERK MAPK signaling using our chemical genetic screen. ACA-28 and GT-7 have unique properties to suppress cell proliferation and induce cell death by further activating ERK in cancer cells with high ERK activity, such as melanoma and pancreatic cancer cells. However, the detailed mechanism of cell death induction by these compounds has not been elucidated. To determine protein targets of ACA-28 relevant for apoptosis induction, we searched for molecules that can bind to ACA-28 and found that BRCA1-associated ATM activator1 (BRAT1), which acts as a DNA damage response protein (DDR protein) upon DNA damage, is a candidate binding protein for ACA-28. We also established a melanoma cell line with acquired resistance to GT-7 (referred to as ACA-R-SK). Interestingly, the expression levels of BRAT1 were significantly higher in ACA-R-SK cells as compared with the original SK-MEL-28 cells. Furthermore, the addition of GT-7 markedly decreased the expression of BRAT1 in both cell lines. Knockdown of BRAT1 by introducing BRAT1 siRNA into the ACA-28 resistant ACA-R-SK cells enhanced cell death induction by GT-7. These results suggest that the down-regulation of BRAT1 may play a key role in the mechanism of cell death induction by GT-7. In this presentation, I will discuss the possible mechanisms of BRAT1 downregulation by GT-7 and the role of BRAT1 in the induction of cell death by GT-7.

References

1) Satoh et al. Identification of ACA-28, a 1' -acetoxychavicol acetate analogue compound, as a novel modulator of ERK MAPK signaling, which preferentially kills human melanoma cells. Genes Cells 2017, 22, 608-618

2) Khandakar et al. ACAGT-007a, an ERK MAPK Signaling Modulator, in Combination with AKT Signaling Inhibition Induces Apoptosis in KRAS Mutant Pancreatic Cancer T3M4 and MIA-Pa-Ca-2 Cells. Cells 2022,11,702

# Exploring responsible molecules increased in blood circulation, which control subject well-being

Higashiyama Ayaka[1,2], Yuna Inada[2], Chihiro Tohda[2]

*[1]Section of Neuromedical Science, Institute of Natural Medicine, University of Toyama, [2]Section of Neuromedical Science, Institute of Natural Medicine, University of Toyama*

Subjective well-being (SWB) is an important research topic being addressed from a variety of perspectives, including psychology, public health and medicine. SWB is influenced by physical activity and cognitive activity, and vice versa SWB affects physical activity and cognitive activity, suggesting that the locomotor system and the brain are closely related to SWB. However, the molecular basis of these interactions has not been clarified. This study aimed to find responsible molecules to control SWB from the blood circulation connecting the locomotor system and the brain. In experiment 1, we conducted a clinical human study to explore molecules increased SWB level-dependently in plasma. Subjective were healthy elderly people aged 65 and over. To elucidate features of elder people with high SWB, subjects were divided into 4 groups by their SWB scores, plasma proteins in 4 groups were comprehensively compared by analysis. As results, the level of protein X increased in high SWB subjects. In experiment 2, mice were bred in enriched environment or stressful environment for 8 weeks to boost positive or negative emotion. Expression levels of protein X in the brain, the skeletal muscle and plasma are quantified. Functional roles of protein X in those organs are also under investigation.

3-B-SS13-1 学生セッション（口頭）

# Arcadlin induction reduces dendritic spine density in the hippocampal dentate gyrus following cerebral ischemia

Nakazawa Shuma, Yosuke Inoue, Shota Inoue, Natsumi Yamaguchi, Jin Nakatani, Toshinori Sawano, Hidekazu Tanaka

*Lab. Pharmacol., Dept. Life Sci., Ritsumeikan Univ.*

Arcadlin, a non-clustered protocadherin δ2, is induced in a neuronal activity and leads to reduction in the dendritic spine density. Cerebral ischemia induces the neuronal activity and the change of dendritic spine morphology. However, the expression of Arcadlin and its role in the ischemia remains unclear. We analyzed Arcadlin expression pattern and dendritic spine changes after cerebral ischemia using a reliable mouse model of middle cerebral artery occlusion (MCAO). We found that *Arcadlin* mRNA was dramatically upregulated in the dentate gyrus (DG) at 4 hours after MCAO. In the ipsilateral DG, we observed a reduction in the dendritic spine density compared to sham mice. However, the MCAO-induced reduction of dendritic spine density was partly mitigated. These findings imply that Arcadlin plays important role in the process of dendritic spine reduction in the DG following cerebral ischemia.

3-B-SS13-2 学生セッション（口頭）

# The PAD4-specific inhibitor GSK484 affords neuroprotection in neonatal hypoxic ischemic brain injury

Xiaoping Yu[1,2], Kai Le[2]

*[1]Nanchang Univ. ･The First Clinical Med. Coll., [2]The First Affiliated Hosp. of Nanchang Univ. ･Dept. of Rehabilitation Med.*

OBJECTIVE: To investigate whether the PAD4 specific inhibitor GSK484 ameliorates brain injury and neurological deficits by inhibiting NETs formation after HIBI and explore the underlying mechanisms.
METHODS: The classical Rice-Vannucci method was used to generate HIBI model. Mice were intraperitoneally injected with GSK484, and the administration time points were 48, 24, 0 h before HI induction and 24, 48 h after HI induction. The mice were divided into three groups: Sham, HI + Saline, and HI + GSK484. MWM, OF and EPM test were used to assess changes in cognitive and psychiatric function in mice. HE and Nissl staining were used to observe the pathological changes in brain tissue and neuronal structural damage. qRT-PCR was used to detect inflammatory factors and chemokines changes. WB and IF were used to demonstrate the expression levels of proteins associated with NETs.
RESULTS: GSK484 reversed HI insult-induced neurobehavioural deficits and the severity of brain injury; The results of QP revealed that the expression of inflammatory factors peaked in brain after 24 h of HI insult, but decreased substantially after the administration of GSK484; HE and Nissl staining showed that GSK484 ameliorated the morphological and structural deformation of brain and neuronal damage induced by HI insult. The mRNA level of chemokines increased significantly after HI insult, and GSK484 treatment reversed this change, suggesting GSK484 reduced neutrophil infiltration into brain; WB and IF indicated that GSK484 could significantly reduce the expression level of NETs-related proteins after HI insults, predicting that the mechanism of neuroprotective action of GSK484 may be through inhibition of NETs formation.
CONCLUSIONS: NETs promote neuroinflammation and brain dysfunction after HI insult, and GSK484-mediated pharmacological inhibition of NETs may provide neuroprotection. These data confirm that the PAD4-specific inhibitor GSK484 may be a potential therapeutic candidate for HIE.

3-B-SS13-3 学生セッション（口頭）

# Neuroprotective effects of MA-5, a mitochondrial activator on cerebral ischemia/reperfusion injury

Shinomi Sasaibe, Yukie Yoshioka, Shinsuke Nakamura, Masamitsu Shimazawa

*Dept. Biofunctional Evaluation., Gifu Pharmaceut. Univ.*

Cerebral ischemia is a lethal disease that causes irreversible neuronal damage and severe sequelae worldwide. Reperfusion therapy is performed after cerebral ischemia but induces further injury with mitochondrial dysfunction. Minimizing ischemia-reperfusion injury and improving prognosis may benefit from mitochondrial protection. In the present study, we investigated the effects of Mitochonic acid -5 (MA-5), which activates mitochondria and increases the efficiency of adenosine triphosphate production, on neuronal cell damage using *in vivo* and *in vitro* experimental models. Male ddY mice were subjected to transient cerebral ischemia by reperfusion 2 hours after middle cerebral artery occlusion (MCAO). Immediately after reperfusion, MA-5 was administered intracerebroventricularly at 0.66 or 0.066 μg. The human neuroblastoma cell line, SH-SY5Y was exposed to oxygen-glucose deprivation followed by reoxygenation (OGD/R). MA-5 significantly reduced infarct volume and improved neurological deficits 24 hours after MCAO. MA-5 suppressed cell death and reactive oxygen species (ROS) production in SH-SY5Y cells after OGD/R. In conclusion, MA-5 has a neuroprotective effect against cerebral ischemia/reperfusion injury, and it might contribute to the improvement of prognosis as a novel therapeutic drug.

3-B-SS13-4 学生セッション（口頭）

# LPS from *P. gingivalis* enhances inflammatory responses of microglia during exposure to amyloid beta

Gui Shuge[1], Zhou Wu[2,3], Tomomi Sano[2], Takashi Kanematsu[2]

*[1]Dept. Oral Maxillofac. Surg. Kyushu Univ., [2]Dept. Cell Biol., Aging Sci. Pharmacol., Kyushu Univ., [3]OBT Research Center, Kyushu Univ.*

【Background】 Brain amyloid beta (Abeta) is accumulated from 20 years before Alzheimer's disease (AD) onset, and microglia is implicated in promoting AD pathogenesis. LPS from *P. gingivalis* (PgLPS), the periodontal bacteria, is detected in AD brain. 【Aim】 In this study, we test our hypothesis that PgLPS enhances microglia-mediated neuroinflammtion in Abeta exposure environment.【Methods & Results】 MG6 microglial cells were exposed to PgLPS (0.1 ug/mL), $Abeta_{1-42}$ (Abeta, 0.1uM) or co-exposed to Abeta (0.1 uM) and PgLPS (0.1 ug/mL). Inflammatory mediators and NFkB signaling were examined. In comparison to control MG6 cells, mRNA expressions of TNF-α, IL-1beta and IL-6 were induced from 1 h after exposure to PgLPS but not Abeta. Interestingly, mRNA expressions of TNF-α, IL-1beta and IL-6 were significantly increased in Abeta and PgLPS co-exposed (AL) MG6 cells from 3 h in compassion to those in PgLPS-exposed cells. TNF-α production were significantly elevated in AL-exposed MG6 cells from 3 h in compassion to that in PgLPS-exposed ones. Furthermore, phosphorylation of IκB and nuclear translocation of p65 NF-κB were up-regulated in PgLPS-exposed MG6 cells but not that in Abeta-exposed cells. Phosphorylation of IκB and nuclear translocation of p65 NF-κB were significantly up-regulated in AL-exposed MG6 cells in compassion to that in PgLPS-exposed cells. 【Conclusion】 The observations strongly suggest that PgLPS enhances microglia-related neuroinflammtion in Abeta exposure environment. The present study provides a new mechanism of periodontitis involving in the early pathologies of AD.

3-B-SS13-5 学生セッション（口頭）

# Elucidation of the Immune Environment and Involvement of Astrocytes in Metastatic Brain Tumors

Sato Keitaro[1], Hiroaki Kato[1], Toya Okawa[1], Goto Momoko[1], Mao Watanabe[1], Chika Matsumoto[2], Hiroki Tanaka[2], Hidetaka Akita[2], Akihiro Hisaka[1], Hiromi Sato[1]

*[1]Clinical Pharmacology & Pharmacometrics, Grad. Sch. Pharmaceut. Sci., Chiba Univ.,, [2]DDS Design and Drug Disposition, Grad. Sch. Pharmaceut. Sci., Tohoku Univ.*

The immune environment of metastatic brain tumors (BrM) remains unknown but is likely to be immunosuppressive with drug resistance. We aimed to determine the immune environment of BrM and the influence of peritumoral astrocytes on it.

Mouse melanoma B16 4A5 was injected into the carotid artery of mice to induce BrM. Finally, the composition of myeloid immune cells in the brain was confirmed by flow cytometry. To recapitulate peritumoral astrocytes in vitro, 2'3'-cyclic AMP-GMP (cGAMP), which is reported to be delivered from BrM to astrocytes and contribute to cancer progression, was introduced directly into astrocytes. The molecular changes downstream of STING signaling that accepts cGAMP were examined. Further, we evaluated the effects of culture supernatants of cGAMP-transfected astrocytes on the migratory ability of neutrophils (derived from HL-60) .

In the brains of BrM, the ratio of neutrophils and macrophages was increased. cGAMP-transfected astrocytes induced and NF-kB target CCL5, and the culture supernatant enhanced HL-60 migration. These results suggest that a specific immune environment is constituted in BrM by mobilizing immune cells of peripheral origin and that astrocyte secretions may be involved.

3-B-SS13-6 学生セッション（口頭）

# Exploration of novel therapeutic agents for adenosine deaminase 2 deficiency using a larval zebrafish.

Hikaru Ishisaka[1], Masahito Sawahata[2], Daisuke Uta[2], Toshiaki Kume[2]

*[1]Dept. Appl. Pharmacol, Grad. Sch. Med. & Pharmaceut. Sci, Univ. Toyama,[2]Dept. Appl. Pharmacol, Grad. Sch. Med. & Pharmaceut. Sci, Univ. Toyama*

Adenosine deaminase 2 (ADA2) deficiency (DADA2) is a hereditary autoinflammatory disease for which no treatment has been established. The reason why exploration of pathogenesis and therapy for DADA2 are difficult is because mice, are often used as disease model animals, have not Cecr1 gene, encoding ADA2. While, zebrafish have two paralogs such as cecr1a and cecr1b, especially cecr1b is considered as DADA2 disease gene. This study aim was to develop the larval zebrafish model for DADA2 therapy. Cecr1b was knocked down (KD) with morpholino antisense oligonucleotide (MO), then cecr1b mRNA was significantly decreased compared with control. Cecr1b-KD zebrafish showed cerebral hemorrhage and motor dysfunction. Inflammatory cytokines have a possibility to be involved in vasculitis and cerebral hemorrhage in patients with ADA2 deficiency. Therefore, we investigated the effect of dimethyl fumarate (DMF), which has anti-inflammatory and antioxidant properties, on DADA2. DMF reduced the rate of cerebral hemorrhage and improved locomotor activity, and significantly decreased the mRNA levels of il-1$\beta$ and il-6. These results suggest that cecr1b-KD zebrafish is a useful model for drug-evaluation system of DADA2 and DMF is a potential therapeutic agent for DADA2.

3-B-SS13-7 学生セッション（口頭）

# Effect of aromatic-turmerone analogues on the activities of chaperone-mediated autophagy and microautophagy

Motomura Kensuke[1], Boateng Alex[2], Masaharu Sugiura[2], Yuki Kurauchi[1], Hiroshi Katsuki[1], Takahiro Seki[3]

*[1]Dept Chemico-Pharmacol Sci, Grad Sch Pharm Sci, Kumamoto Univ, [2]Grad Sch Pharm Sci, Sojo Univ, [3]Dept Pharmacol, Fac Pharm Sci, Himeji Dokkyo Univ*

Autophagy-lysosome proteolysis regulates protein homeostasis in neurons and is classified into macroautophagy, microautophagy (mA), and chaperone-mediated autophagy (CMA). Among them, we focused on CMA and mA and established a novel method to monitor CMA/mA activity. Recently, we identified an aromatic (ar)-turmerone analog (A2) that protects dopaminergic neurons via the activation of an antioxidant transcription factor, Nrf2, which is known to activate CMA/mA. In this study, we attempted to identify novel ar-turmerone analogs that can activate Nrf2 and CMA/mA more potently than A2. We synthesized four novel ar-turmerone analogues (A4-A7) and investigated their abilities to activate Nrf2 and CMA/mA. Immunoblot experiments revealed that all compounds significantly upregulated Nrf2 in SH-SY5Y cells. In contrast, only A4 significantly activated CMA/mA. To investigate why these analogs differently affected CMA/mA activity, we focused on p38 that regulates CMA via the phosphorylation of LAMP2A. Among ar-turmerone analogs that we investigated, only A4 induced the persistent activation of p38. In addition, experiments using inhibitors revealed that CMA/mA activation by A4 is mainly mediated by p38 activation. Taken together, we identified a novel ar-turmerone analog (A4) that activate CMA/mA via the sustained activation of p38.

3-B-SS14-1 学生セッション（口頭）

# Differential toxicity and localization of arginine-rich C9ORF72 dipeptide repeat proteins depend on de-clustering of positive charges

Tamami Miyagi, Kohsuke Kanekura

*Department of Pharmacology, Tokyo Medical University*

Recently, many Amyotrophic lateral sclerosis (ALS)-causing proteins reportedly undergo liquid-liquid phase separation (LLPS). It has also been shown that arginine-rich dipeptide repeat proteins (R-DPRs), poly(PR) and poly (GR), expressed from mutant C9ORF72 are prone to phase separation, and associate with membrane-less organelles formed by LLPS, disturbing their functions. Although R-DPRs share many biochemical features, such as the alternating of Arg, their subcellular localization and toxicity mechanisms are different. However, the mechanisms underlying these differences are unknown. In this study, we analyzed localization, intermolecular interactions, and LLPS of R-DPR variants, and found that these differences are determined by the degree of segregation of Arg charges (Miyagi et al., iScience 2023). In poly(PR), the segregation of arginine side chains by Pro is a critical factor in promoting nucleolar incorporation. In addition, Pro allowed interactions with molecules in a weak but highly multivalent manner. Still, Gly was incapable of separating the Arg well, so poly(GR) showed static localization in the cytosol. Poly(GR) binds to molecules in a strong, but less multivalent manner than poly(PR). These data reveal critical factors that determine biochemical differences in the R-DPRs.

3-B-SS14-2 学生セッション（口頭）

# Involvement of tryptophan metabolism in the pentylenetetrazol-induced epileptic seizures of mice

Takaya Nishikawa[1], Akihiro Mouri[1,6], Kazuo Kunisawa[1], Masaya Hasegawa[1], Shuhei Yamagishi[1], Takatoshi Sakata[1], Tomoya Sugai[2], Noriki Kutsumura[2,3], Kuniaki Saito[4,5], Toshitaka Nabeshima[5,6]

*[1]Dept. Regulatory Sci., Grad. Sch. Health Sci., Fujita Health Univ., [2]International Institute for Integr. Sleep Med. (WPI-IIIS), Univ. of Tsukuba, [3]Grad. Sch. of Pure and Appl. Sci., Univ. of Tsukuba, [4]Dept. Disease Control & Prevention., Grad. Sch. Health Sci., Fujita Health Univ., [5]Lab. Health & Med. Sci. Innov., Grad. Sch. Health Sci., Fujita Health Univ., [6]NPO. J-DO*

Epilepsy induces seizures as the result of excessive electrical excitation in the brain. The excitation-inhibition balance (EI balance) of the central nervous system (CNS) is implicated in the pathophysiology of epilepsy. The tryptophan pathway generates several metabolites which modulate glutamatergic neuronal system such as kynurenic acid (KA: NMDA receptor glycine-site antagonist) and quinolinic acid (QA: NMDA receptor agonist). We investigated whether alterations of tryptophan metabolism contribute epileptic seizures by disrupting the EI balance in the CNS. KA attenuated pentylenetetrazol (PTZ)-induced epileptic seizures, but QA exacerbated it. Chronic administration of PTZ exacerbated epileptic seizures and increased expression of kynurenine 3-monooxygenase (KMO, which involved in QA synthesis), but decreased expression of quinolinate phosphoribosyl transferase (QPRT, which involved in QA metabolism) and kynurenine amino transferase (KAT, which involved in KA synthesis). Seizure exacerbation was suppressed in KMO heterozygous knockout mice but exacerbated in QPRT knockout mice. These data suggested that expression changes of tryptophan metabolic enzymes may exacerbate PTZ-induced epileptic seizures, through change in the metabolic balance between KA versus QA.

3-B-SS14-3 学生セッション（口頭）

# Pharmacological effects of bioactive phospholipids on cellular dysfunction caused by seed-dependent alpha-synuclein aggregation

Furukawa Masaya[1], Tamotsu Tsukahara[1], Miku Tanaka[1], Masanori Sasaki[1], Yoshikazu Matsuda[2], Hisao Haniu[3]

*[1]Dept. of Pharmacol. and Therap. Innov., Nagasaki Univ. Grad. Sch. of Biomed. Sci., [2]Div. of Clin. Pharmacol. and Pharmaceutics, Nihon Pharmaceut. Univ., [3]Inst. for Biomed. Sci., Interdiscip. Cluster for Cutting Edge Res., Shinshu Univ.*

Alpha-synuclein（α-Syn）aggregation is known to be a causative factor in synucleinopathies, including Parkinson's disease and dementia with Lewy bodies, but the detailed mechanism and treatment remain unresolved. In addition, various cellular dysfunctions have been reported to occur in brain cells of patients with synucleinopathy. We are investigating the pharmacological effects of lysophospholipid (LPL) extracted from porcine liver enzyme degradation product (PLDP) on these cellular dysfunctions. PLDP is used in functional foods and has been reported to improve human cognitive function and inhibit intracellular α-Syn aggregation in previous studies. In this experiment, we use an α-Syn aggregating cell model in which an α-Syn stable expression line was generated using human neuroblastoma SK-N-SH and transfected with α-Syn seeds. This model was exposed to LPL and seed-dependent cytotoxicity was evaluated, and the ameliorative effect of LPL was confirmed. The purpose of this study is to verify whether LPL has an ameliorative effect on cellular dysfunction and to expand its potential as a novel therapeutic agent for synucleinopathy.

3-B-SS14-4 学生セッション（口頭）

# Ciliary rootlet morphology shows distinct pattern in each region of mouse brain

Akinori Takayama, Kenshiro Uemura, Nana Koyama, Kaito Hannoe, Miu Hayashi, Zhongyang Xiao, Jin Nakatani, Toshinori Sawano, Hidekazu Tanaka

*Crs. Biomed., Grad. Sch. Life Sci., Univ. Ritsumeikan.*

Primary cilia are short protrusions that are present in most cells and function as an extracellular environment sensor. In some tissues, a supporting cytoskeleton called the ciliary rootlet (CR) extends from the base of the primary cilium toward the interior of the cell. Dysfunction of primary cilia causes ciliopathies that lead to serious disruption of brain tissue structure and function, but there is no effective treatment for ciliopathies. Recent studies reveal the molecular mechanisms that cause dynamic changes in primary cilia providing the bases for the development of therapeutic drugs. However, CRs in the CNS have not been well studied; their morphology or function are still unknown. Here, we revealed that CRs in C57BL/6 mouse brain are abundant, and their morphology shows distinct pattern in each region. CR morphology in the CNS was roughly classified into three types: linear, circular, and fluffy. The ratio of linear CRs decreased and that of circular CRs increased along with the mouse development. Generally, the CRs were located on the apical dendritic side of cell bodies. Interestingly, there was a positive correlation between CRs length and cilia length, that is, longer cilia are equipped with longer CRs in the brain. The clarification of characteristics of the CRs should lead to a more detailed understanding of the molecular mechanisms of cilia function and to the development of treatments for ciliopathies.

3-B-SS14-5 学生セッション（口頭）

# Dexmedetomidine alters hippocampal astrocyte morphology via $\alpha_2$-adrenoceptor *in vivo.*

Watanuki Shu[1], Taisuke Kitano[2], Ryota Eguchi[1], Kohei Morimoto[1], Ken-ichi Otsuguro[1]

*[1]Lab. Pharmacol., Fac. Vet. Med., Hokkaido Univ.,[2]Lab. Vet. Biochem., Sch. Vet. Med., Kitasato Univ.*

Astrocytes have complex structures with numerous branches, and their morphology is related to the CNS functions. In our laboratory, we have previously showed that activation of $\alpha_2$-adrenoceptor (AR) inhibits the process formation of cultured astrocytes. However, the effects of dexmedetomidine (DEX), an $\alpha_2$-AR agonist, on the astrocyte morphology *in vivo* remain unclear. Therefore, we investigated the impact of DEX on astrocyte morphology *in vivo*.

Mice were intraperitoneally injected with $\alpha_2$-AR agonist DEX (1-100 μg/kg) and/or $\alpha_2$-AR antagonist atipamezole (ATIP) (1 mg/kg). GFAP was stained on brain slices, and morphological parameters were evaluated using SMorph. Additionally, lucifer yellow (LY) was injected into individual astrocytes using microelectrodes to evaluate astrocyte volume.

DEX decreased surface area of GFAP cytoskeleton, length, and thickness of astrocyte processes. Morphological analysis using LY similarly showed a reduction in astrocyte volume without changes in cell body size. ATIP partially inhibited astrocyte morphological changes by DEX.

This study showed that DEX induces morphological change of astrocyte, primarily mediated by $\alpha_2$-AR. However, since ATIP did not completely inhibit the morphological changes induced by DEX, other pathways, including the receptors other than $\alpha_2$-AR, may be involved in it.

3-B-SS14-6 学生セッション（口頭）

# Unveiling Neuro-behavioral and -histological changes in mice lacking three genes in the human 3p26.3 locus

Shindo Asuka[1], Moka Tsuchiya[1], Nozomi Tanaka[1], Asami Oguro-Ando[2], Eri Segi-Nishida[1]

*[1]Dept of Biol Sci and Tech, Fac of Adv Eng, Tokyo Univ of Sci,[2]Univ of Exeter Med Sch, Dept of Clin and Biomed Sci*

3p-deletion syndrome, characterized by the absence of human chromosome 3p, often reflects Autism Spectrum Disorder (ASD)-like behaviors. Notably, genes of interest, CHL1, CNTN6, and CNTN4, nestled within the genomic locus 3p26.3, are implicated in ASD risk. This study embarks on deciphering the intricate mechanisms orchestrating behavioral and neural shifts. Through transgenic mice lacking these genes (3pKO), we seek to unravel these complexities. To evaluate social ability, anxiety and repetitive behavior, we conducted social interaction test (SIT), Open field test (OFT), and measuring grooming total time. The result in SIT showed that control mice spent about 70% of approaching time for the social partner, but it was about 50% in 3pKO mice, indicating 3pKO mice decreased socialization. The result in OFT showed 3pKO mice spent significantly more time in the central area and the grooming total time was decreased in 3pKO mice, indicating reducing anxiety and repetitive behavior. In addition, histological analysis of brain sections by nissl staining revealed morphological abnormalities in the hippocampus and enlargement of the lateral ventricle in some individuals. We now examine the expression levels of genes related to neurogenesis and autism-related genes in the hippocampus. As we carefully analyze the complex interactions between behavioral, histological, and expression changes in 3pKO mice, a comprehensive understanding unfolds. The significance of CHL1, CNTN6, and CNTN4 genes in shaping hippocampal function and social behaviors urges us to untangle the intricate processes at play, uncovering the underlying scientific mechanism.