1-O-001 Oral Sessions

Important role of neuronal histamine N-methyltransferase in brain functions

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Histamine plays an important role in the control of brain functions. Our recent study showed that histamine *N*-methyltransferase (HNMT), a histamine-metabolizing enzyme, regulates brain histamine concentration. Although previous study indicated the expression of HNMT in neurons, the contribution of neuronal HNMT to brain histamine system is still unknown. In the present study, we phenotyped neuron-specific Hnmt knockout (cKO) mice to clarify the importance of neuronal Hnmt. First, we generated cKO mice by injecting AAV9-hSyn1-Cre, which express Cre recombinase specifically in neurons, to Hnmt flox mice. Histamine concentration of cKO brains was significantly elevated compared to that of control brains, indicating the importance of neuronal Hnmt for brain histamine concentration. Behavioral test battery demonstrated that cKO mice showed reduced anxiety-like behaviors, reduced depression-like behaviors and increased locomotor activity in novel and familiar environment. These data show the importance of neuronal Hnmt for brain histamine system and brain functions.

5-HT_{1A} and 5-HT_{1B} receptor-mediated inhibition of excitatory synaptic transmission onto rat basal forebrain cholinergic neurons

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A whole-cell patch-clamp study was carried out to elucidate serotonin (5-HT)-induced modulation of excitatory synaptic transmission onto cholinergic neurons in the basal forebrain (BF) using brain slices obtained from young rats (P12-20). BF cholinergic neurons were identified with Cy3-192IgG. Excitatory postsynaptic currents (EPSCs) were evoked by focal stimulation. 5-HT, 8-OH-DPAT (DPAT), a 5-HT_{1A} receptor agonist or CP93129 (CP), a 5-HT_{1B} receptor agonist inhibited the amplitude of EPSCs. 5-HT-induced inhibition was mostly antagonized in the presence of both 5-HT_{1A} and 5-HT_{1B} receptor antagonists. Paired-pulse ratio (PPR) and coefficient of variation (CV) of the EPSCs were increased by CP, whereas DPAT had no effect on PPR or CV. DPAT inhibited the inward currents induced by puff application of L-glutamate, whereas CP had no effect. 5-HT-induced inhibition was decreased in the presence of ω-agatoxin TK (Aga) compared to that without Aga. Furthermore, CP-induced inhibition of EPSCs was eliminated in the presence of Aga, whereas DPAT still inhibited the EPSCs even in the presence of Aga. These results suggest that 5-HT_{1A} receptors reduce the sensitivity of postsynaptic glutamate receptors, whereas 5-HT_{1B} receptors presynaptically inhibit glutamate release by selectively blocking P/Q-type calcium channels, thereby both inhibiting excitatory transmission onto BF cholinergic neurons.

Involvement of 8-nitro-cGMP - ERK signals in the induction of long-term depression in the mouse cerebellum

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Long-term depression (LTD) at parallel fiber to Purkinje cell (PC) synapse in the cerebellum is the cellular basis for cerebellar motor learning. Although some signaling molecules including protein kinase G (PKG) and MAP kinase (MAPK) are essential for the LTD induction, the molecular mechanism for long-term memory has not been fully understood.

8-nitro-cGMP is produced by guanylyl cyclase in the presence of nitric oxide and reactive oxygen species (ROS). In contrast to cGMP, 8-nitro-cGMP has resistance to PDE-dependent catalysis and can activate PKG for a long time. Therefore, we hypothesized that 8-nitro-cGMP-mediated signal is essential for cerebellar LTD. Application of 8-nitro-cGMPS, an analog for 8-nitro-cGMP, to PCs significantly inhibited LTD induction in acute mouse cerebellar slices. Pharmacological inhibition of ROS also abolished LTD induction, suggesting involvement of ROS/8-ntiro-cGMP signals in cerebellar LTD.

8-nitro-cGMP activates protein kinase G (PKG), and our previous studies indicate PKG activate extracellular signal related kinase (ERK), a type of MAPK. We therefore examined involvement of ERK in cerebellar LTD, using mutant mice lacking ERK 1 and/or 2. The LTD was impaired in the ERK 1&2 double-knockout cerebellum. There results indicate essential role of 8-nitro-cGMP – ERK signals in cerebellar LTD.

Development of a method for single-molecule imaging in the brain tissue

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Accumulating evidence suggests that molecular dynamics at nanometer scale is crucial for brain functions and disorders. Single-molecule fluorescence imaging is a super-resolution live imaging method that enables direct tracking of movement of individual molecules. However, conventional single-molecule imaging has been applicable only to dissociated cells on coverslips due to technical limitations, preventing the analysis of events that occur only in the intact brain tissue. In this study, we set out to develop a method for single-molecule imaging within brain slices and the brain *in vivo*. We developed and employed a novel chemical tag technology named De-QODE. This technology consists of a small-molecular QODE probe and DeQODE protein tag. Non-fluorescent QODE becomes highly fluorescent upon reversible binding to DeQODE. These properties allow us to lower background and avoid photobleaching even in light-scattering tissue samples. We succeeded in continuous and high-density tracking of QODE molecules activated by membrane-tagged DeQODE in pyramidal neurons deep within acute cortical slices. This result indicates that our De-QODE-based method is highly promising to realize the pharmacology based on single-molecule dynamics.

A fluorescent sensor for the real-time measurement of extracellular oxytocin dynamics in the brain

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Oxytocin (OT), a hypothalamic neuropeptide that acts as a neuromodulator in the brain, orchestrates a variety of animal behaviors. However, the relationship between brain OT dynamics and complex animal behaviors remains largely elusive, partly because of the lack of a suitable technique for its real-time recording *in vivo*. Here, we describe a G protein-coupled receptor-based green fluorescent OT sensor with a large dynamic range, optimal affinity, ligand specificity to OT orthologs, minimal effects on downstream signaling, and long-term fluorescence stability. By combining viral gene delivery and fiber photometry-mediated fluorescence measurements, we demonstrated the utility of the sensor for real-time detection of brain OT dynamics in living mice. Importantly, our measurements revealed "OT oscillation," a hitherto unknown rhythmic change in OT levels in the brain. The new fluorescent OT sensor will allow the analysis of OT dynamics in a wide variety of physiological and pathological processes.

Hepatocellular carcinoma induces tissue osmolyte and water retention, and body mass loss in rats

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Liver dysfunction including liver cirrhosis and hepatocellular carcinoma (HCC) often causes osmolyte and water imbalance such as edema and ascites. However, the feature and mechanism of liver injury-mediated body fluid dysregulation remain to be elucidated. In the present study, we examined the effect of HCC on body sodium and water balance in rats. Male Wistar rats were administered diethylnitrosamine, a carcinogenic drug, for 8 weeks to establish HCC. Three weeks after the cessation of diethylnitrosamine administration, we evaluated body mass, sodium, and water balance. Compared with control rats, HCC rats reduced body mass and the amount of total body sodium and water. HCC rats also showed enhanced glucocorticoid receptor activity in skeletal muscle, a marker of catabolism. On the other hand, relative tissue sodium and water content per skin and muscle tissue weight were significantly increased in HCC rats. These HCC-induced changes in sodium and water balance were significantly associated with increased 24 hours urinary aldosterone excretion and increased urea osmolyte-driven renal water conservation. These findings suggest that HCC induces osmolyte and water retention at the tissue level in parallel with body mass loss and that enhanced glucocorticoids, aldosterone, and the urea-driven renal water conservation system lead to these HCC-induced changes. The tissue sodium and water retention accompanied by body mass loss may be a causative factor for osmolyte and water imbalance in liver failure.

1-O-007 Oral Sessions

Anemia disrupts renal compensatory growth without paralysis of growth signaling pathway

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Kidney has ability to compensate its size and function against the nephron loss for maintaining total renal function, for example, in both donor and recipient in renal transplantation. However, the factors that regulate this compensation have not been fully clarified yet. It has been reported that approximately 70% of renal transplantation recipients suffer from anemia. Hereby we examined the effects of anemia on the compensatory renal hypertrophy in the mice lacking erythropoietin production. The anemic mice showed disrupted compensation after UNX compared to normoxemic mice. The disruption was accompanied by the sustained phosphorylation of ribosomal protein S6, a marker of mTOR activation, and by the sustained activation of YAP, a key transcriptional factor for the organ development; both of which had been normalized after successful compensation in the normal mice. There were no difference in the numbers of Ki67- and TUNEL-positive cells and in the capillary blood flow between anemic and normoxemic mice. In conclusion, anemia disrupted compensatory renal hypertrophy after UNX despite the activated tissue growth signals.

1-O-008 Oral Sessions

Distal tubular NCX1 plays a critical role in ischemic acute kidney injury

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Ischemic acute kidney injury (AKI) is a serious renal dysfunction caused by surgical invasion and transplantation. Proximal tubular damage is known to be the main characteristics of pathological progression in ischemic AKI. However, the involvement of distal tubule in ischemic AKI remains well unknown. We have previously shown that Na^{+}/Ca^{2+}exchanger type 1 (NCX1) is expressed on the basolateral side in distal convoluted tubule and is involved in urine production and electrolyte exertion. In this study, we evaluated the pathophysiological significance of distal tubular NCX1 in ischemic AKI, using distal tubular-specific NCX1 deficient (NCX1-cKO) and NCX1 transgenic (NCX1-TG) mice. We subjected these mice and wild-type mice to sham surgery or 30 min of unilateral renal ischemia and reperfusion (IR) with contralateral nephrectomy. BUN and serum creatinine were significantly increased in both NCX1-TG mice and wild-type mice after IR injury. In contrast, these increases were remarkably suppressed in NCX1-cKO mice after IR injury. The histological findings showed that the distal tubular damages after IR injury were alleviated in NCX1-cKO mice, but were exacerbated with macrophage infiltration to renal medulla in NCX1-TG mice. These results suggest that distal tubular NCX1 plays a critical role in the pathophysiology of ischemic AKI.

The effects of TRPC6 activation by mechanical and receptor stimulations on podocyte-mediated filtration barrier function

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Sustained mechanical stresses (e.g. high blood pressure) damage the renal glomerulus resulting in proteinuria. Podocytes express the canonical transient receptor potential 6 (TRPC6) channel, the activity of which is modulated by receptor and mechanical stimulations in a complex manner. However, the implication of this modulation for the glomerular barrier function is rather poorly elucidated. To address this point, we carried out the albumin-permeation assay using immortalized mouse podocytes stably expressing the wild-type (wt) TRPC6 or its gain-of-function mutant associated with focal segmental glomerulosclerosis (FSGS), M131T. Differentiated podocytes were grown on cell-culture insert membranes (pore size: 0.4micrometer). Compared to unstimulated or angiotensin II (Ang II) alone, simultaneous stimulation with Ang II and a membrane-expanding agent 2,4,6-trinitrophenol (TNP) reduced the leak of FITC-labelled albumin across the membranes in both wt-TRPC6- and M131T-expressing podocytes. SAR7334, a potent TRPC6-specific inhibitor, increased the albumin leak, the effect being more prominent in the latter. These results suggest that TRPC6 activation by simultaneous receptor and mechanical stimulations may reinforce the filtration barrier function mediated by podocytes, and this may be impaired by the FSGS-associated mutation M131T.

IL-6 family cytokines, STAT3 activators, exhibit differential effects in a ligand-specific manner in podocytes

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[Background]
IL-6 family cytokines play protective roles in cardiomyocytes via STAT3 activation. Glomerular podocytes, as an important component of the kidney blood filtration, are terminally differentiated cells as well as cardiomyocytes. Although some studies have shown that STAT3 activation is associated with podocyte dysfunction, it remains unclear whether activated STAT3 exhibits differential functions depending on cytokines. The aim of this study is to assess the effects of IL-6 family cytokines/STAT3 signaling in podocytes.
[Methods and results]
To examine the pathophysiological relevance of IL-6 family cytokines in kidney diseases, C57BL/6J mice were subjected to ischemia-reperfusion or lipopolysaccharide (LPS) treatment. Quantitative PCR demonstrated that the expression level of IL-6, leukemia inhibitory factor (LIF) and IL-11 was upregulated in injured kidneys. In cultured podocytes, STAT3 was rapidly activated in response to the stimulation with IL-6, LIF or IL-11. Interestingly, LIF and IL-11 treatment suppressed H_2O_2-induced cell death in cultured podocytes, whereas IL-6 tended to increase cell death.
[Conclusion]
STAT3 could differentially function in an activator cytokine-specific manner, in podocytes, providing the important information for the development of therapy targeting STAT3 for kidney diseases.

Distinct downstream targets of the medial prefrontal cortex underlie discrete antidepressant responses to ketamine

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Ketamine, an N-methyl-D-aspartate (NMDA) receptor antagonist, is the prototype for a potential new generation of glutamate-based antidepressants that rapidly relieve symptoms of depression within hours of treatment. Studies in rodents have demonstrated that neuroplasticity in the medial prefrontal cortex (mPFC) is critical for the antidepressant actions of ketamine. However, effector circuits downstream of the mPFC underlying the rapid antidepressant responses remain unknown. To address this issue, we used optogenetic and chemogenetic circuit mapping in rodent models for studying depression, demonstrating the role of the basolateral amygdala (BLA) and bed nucleus of stria terminalis (BNST) as downstream targets of the mPFC mediating distinct behavioral effects of ketamine. By inhibiting isolated mPFC projections in the period immediately following ketamine administration, we found that mPFC-mediated activation of BLA principal neurons, and subsequent projections to the ventral hippocampus, mediate a subset of ketamine's persistent antidepressant-like effects on passive coping behavior but not on anxiety-like and reward-seeking behaviors. In contrast, mPFC projections to the BNST are necessary and sufficient to produce persistent antidepressant-like effects on anxiety-like and reward-seeking behaviors but not on passive coping behavior. Therefore, our data support a model where distinct downstream circuits of the mPFC contribute to producing separate antidepressant-like behavioral responses.

Effects of KNT-127, a delta opioid receptor agonist, on sleep in mice.

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Sleep is closely related with mental health, and affected by drug therapy in psychiatric disorders, and vice versa. The delta opioid receptor (DOR) agonist KNT-127 has been reported to have anxiolytic effects. Unlike benzodiazepines, KNT-127 has no side effects such as dizziness and amnesia. However, its effects on sleep have not been studied. In the present study, we investigated the effects of KNT-127 on sleep in the light period in mice. The vigilance states (e.g., wakefulness, rapid eye movement (REM) and non-REM sleep) of the ddY-mice (6-10 weeks) were classified based on the hippocampal local field potential (LFP) and neck muscle electromyogram. KNT-127 (3-30 mg/kg, i.p.) dose-dependently decreased the mean REM and non-REM sleep period, and prolonged the mean wakefulness period during 5 hr after its injection. At the wakefulness and REM sleep periods after the KNT-127 treatment, the gamma power and theta peak frequency were increased in the hippocampal LFP, suggesting that KNT-127 enhanced the neuronal activities. Pre-treatment of naltrindole (10 mg/kg, s.c.), a DOR antagonist, prevented the KNT-127-induced decrease in non-REM, but not REM, sleep. Naltrindole alone did not influence the vigilance states. Together, KNT-127 increased wakefulness by decreasing non-REM and REM sleep via DOR-dependent and -independent mechanisms, respectively.

Contribution of kynurenine-3-monooxygenase to hemorrhagic neuron injury

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Kynurenine 3-monooxygenase (KMO) is a kind of rate-limiting enzyme in the kynurenine pathway. We investigated the change in KMO expression and intermediary metabolite levels after intracerebral hemorrhage (ICH) in neuronal injury. Treatment with thrombin to primary-cultured microglia increased the KMO expression through the p38 MAPK pathway. In the cultured medium, the ratio of quinolinic acid (QUIN), an N-methyl-D-aspartate receptor agonist, to kynurenic acid (KYNA), its antagonist, was increased, whereas the level of 3-hydroxykynurenine, a redox-active compound, showed no significant change. The increased QUIN/KYNA ratio was blocked by Ro61-8048, a KMO inhibitor. In the mouse ICH model, immunohistochemical staining showed that KMO was co-localized with neurons, microglia, and astrocytes. The QUIN/KYNA ratio was increased after ICH, but blocked by the intracerebroventricular injection of Ro61-8048 or liposomal clodronate, a microglia toxin. Ro61-8048 ameliorated the loss of neurons, as indicated by NeuN-immunopositive cells, at the perihematomal region and repaired their abnormal behaviors without affecting the hematoma size. In conclusion, thrombin-induced alterations of microglial KMO and intermediary metabolites of the kynurenine pathway were suggested to play important roles in neuronal injury after ICH.

The effect of sphingosine related pathway activation in early brain injury after subarachnoid hemorrhage in rat

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It is recently known that early brain injury is one of the important pathophysiology to determinant the prognosis of subarachnoid hemorrhage (SAH). We herein examined the role of sphingosine related pathway activation in the treatment of early brain injury of experimental SAH model.

SAH were induced by endovascular perforation in mice or rats, and they were treated with 1) unfractionated heparin 2) isoflurane 3) FTY720 (sphingosine receptor agonist). The animals evaluated neurological scores, brain edema and sphingosine metabolism-related molecular markers.

The above-mentioned treatments improved neurofunction and brain edema, and provided antiapoptotic effects such as upregulation of phosphorylated Akt and downregulation of caspase-3. The effects were associated with activation of sphingosine kinase and sphingosine receptor.

We suggest that activation of the sphingosine related pathway should have beneficial effects in early brain injury of experimental SAH and could improve the prognosis of the clinical SAH patients.

Inhibitory effects of pine nodule extract on mental stress-induced increases in sympathetic nervous activity in young students

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Pine nodule extract and its component, SJ-2, have been reported to have an inhibitory effect on catecholamine secretion induced by acetylcholine (its physiological secretagogue) in cultured bovine adrenal medullary cells (J Pharmacol Sci., 2017).

The present study aimed to determine the effect of pine nodule extract on the autonomic nervous activity induced by mental stress (Uchida-Kraepelin arithmetic test) in healthy young students. Autonomic nervous balance was measured by power spectral analysis of heart rate variability using a standard hexagonal radar chart. Four 15-min repetitions of an arithmetic task served as an acute mental stressor that caused an increase in sympathetic parameters in the placebo group, while no increases in sympathetic parameters were observed in the pine nodule extract group.

The present study demonstrated that the assay system of the autonomic nervous balance detected increases in sympathetic nervous activity induced by acute arithmetic stress in the placebo group, but that those increases were cancelled in healthy young students who ingested pine nodule extract, suggesting that this pine nodule extract may an anti-stress effect.

TRAb-IgM induced by Epstein-Barr virus reactivation does not have thyroid stimulating effect, but injures the thyroid follicular epithelial cells and releases thyroid antigens

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Thyrotropin receptor antibody (TRAb) is a causative antibody of Graves' disease. Epstein-Barr virus (EBV) persists in human B cells, and occasionally reactivates. We have reported that both Graves' disease patients and healthy controls have EBV-infected B cells that have TRAb as their surface globulin (TRAb(+) EBV(+) cells). The peripheral blood mononuclear cells containing TRAb(+) EBV(+) cells produced TRAbs along with EBV reactivation. We proposed the EBV reactivation-induced Ig production as alternative system of Ig production.

The antibodies produced by EBV reactivation-induced system are IgM dominant, and skewed to be autoreactive. However, the class of thyroid stimulating TRAb is known to be IgG. We studied about the role of the IgM antibody.

We purified TRAb-IgM from culture medium of TRAb(+) EBV(+) cells. Then, we cultured porcine thyroid cells with the TRAb-IgM and complements, and then, measured cAMP and LDH levels to estimate thyroid stimulating effect and cell injury, respectively. We observed the increase of the levels of LDH, but could not detect cAMP.

We considered that TRAb-IgM did not have thyroid stimulating effect, but it could injure the thyroid cells and release thyroid antigens including TSH receptor antigen. The relevance of EBV reactivation to Graves' disease may have a possibility for the new therapy.

Maternal high calorie diet during pregnancy induces abnormal hepatic glycogenolysis in the offspring and it is mitigated by osteocalcin

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Maternal nutrition during pregnancy has been found to have a significant impact upon the health of offspring after maturation. However, strategies for modulation of metabolism without an adverse effect on the fetus have remained limited. It was recently shown that maternal high calorie diet induces obesity in later life of the offspring, and maternal oral administration of uncarboxylated osteocalcin (GluOC), which crosses the placenta, improves metabolic status in the offspring by unknown mechanism.

We thus explored the molecular mechanisms for the effect of maternal high calorie diet and GluOC during gestation on metabolic properties of the offspring. From our results, a maternal high-fat, high-sucrose diet during pregnancy causes metabolic disorders in the liver of the offspring due to hypermethylation of the *Pygl* gene, encoding glycogen phosphorylase L, which mediates hepatic glycogenolysis. The lower expression of Pygl induced by the maternal diet causes the hepatic accumulation of glycogen and triglyceride in the offspring, which remains in adulthood. On the other hand, maternal GluOC upregulates *Pygl* expression in the offspring *via* both direct and indirect pathways to improve maternal diet-induced obesity and abnormal energy metabolism.

We propose that maternal high calorie diet is reflected in the hepatic glycogenolysis capacity of the offspring *via* epigenetic modification of *Pygl* and maternal oral administration of GluOC protects the offspring from metabolic disorders induced by maternal diet by regulation of glycogenolysis.

From zebrafish to clinical: Rhamnan sulfate improves constipation with gut microbiota alteration in double-blind placebo-controlled trial.

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Rhamnan sulfate (RS) is a sulfate polysaccharide composed of L-rhamnose and sulfated L-rhamnose found in green algae such as *Monostroma nitidum*. It has been reported to have anticoagulant and antiviral effects in the last decade. In 2015, our group discovered the anti-obesity effect of RS using the diet-induced obesity model of zebrafish (Zang L, et al. J Funct Foods. 2015;17:364-370). In this study, we first administrated RS (0.25 mg/g food volume) orally to high-fat diet-treated mice for 4 weeks. RS increased fecal volume and calorie excretion with suppression of body weight increase, and decreased plasma lipids and fasting blood glucose levels, which was consistent with the results of our zebrafish study. Noting the increased excretion by RS, we administrated RS (100 mg/day) to subjects with low defaecation frequencies (3–5 times/week) for 2 weeks in a double-blind placebo-controlled manner. As a result, Firmicutes tended to decrease, while Bacteroides increased. It is known that the obese population has more Firmicutes and fewer Bacteroides depending on their body mass index. Thus, RS improved the intestinal microflora in the direction of anti-obesity. In addition, clostridia (Firmicutes), which produce medium-chain fatty acids that increase the absorption of water in the intestine (one of the causes of constipation), were reduced by the intake of RS. Furthermore, Metagenomics profiling using PICRUSt and KEGG pathway showed that RS intake activated the "cytochrome p450-mediated excretion of foreign substances" pathway, "biological defense against invasive bacteria," and the "biomolecular NAD synthesis" pathway, which has already been reported to have a therapeutic effect on constipation, suggesting a possible mechanism for the ameliorating effect of RS.

Characteristics of the adipocytes differentiated by a glucocorticoid receptor antagonist RU486

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The combination of isobutylmethylxanthine, insulin and dexamethasone (MIX treatment) is widely used to differentiate 3T3-L1 fibroblasts to mature adipocytes. In the preliminary experiment performed to determine the role of glucocorticoid receptor, a single treatment with RU486, a glucocorticoid receptor antagonist, was counterintuitively found to be sufficient to induce adipogenesis with smaller lipid droplets than MIX treatment. Here we aimed to characterize the RU486-induced adipocytes by DNA microarray analysis and compare to those in the adipose tissues. Total RNAs isolated from the undifferentiated 3T3-L1 fibroblasts (control cells) and the adipocytes induced by 5-day treatment with 1 micromolar RU486 (RU cells) and those induced by MIX treatment (MIX cells) were subjected to analysis with an Affimetrix Clariom S Mouse DNA chip. The microarray data of the mouse epididymal and inguinal adipose tissues were obtained from GEO DataSet in NCBI, the National Institute of Health, USA. Transcriptome Analysis Console 4.0.2 (Thermo Fischer Scientific) was use to analyze the data. The primary component analysis revealed that the RU cells were closer to epididymal and inguinal adipose tissues in terms of primary component 2 and 3, respectively, than MIX cells or control cells. Forty four genes were found to be common between RU cells and epididymal adipose tissues. The findings indicate RU486 induces adipocytes closer to those of in vivo adipose tissues than MIX treatment. Single treatment with RU486 is suggested to be a novel method to induce normal adipogenesis.

Negative impact of heparan sulfate in hepatocytes on insulin sensitivity

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Heparan sulfate (HS) is a highly sulfated glycosaminoglycan distributed on the cell surface. Our recent studies revealed that HS played an essential role in energy homeostasis through the regulation of insulin secretion from pancreatic β-cells and glucose sensitivity in adipose tissues. Although liver is one of the most important organs for energy homeostasis, the functions of HS in liver remain largely unknown. In the present study, we phenotyped hepatocyte-specific HS-deleted mice (cKO) to elucidate the roles of HS in hepatocytes.

Blood glucose testing showed that HS deletion in hepatocytes resulted in the lower glucose level of cKO after glucose challenge due to higher insulin sensitivity, indicating the augmented insulin signaling in cKO hepatocytes. Indeed, the phosphorylation level of Akt, which is one of the important molecules of insulin signaling, was robustly increased in cKO liver after insulin treatment. Biochemical assays indicated HS reduction led to the enhanced differentiation of hepatocytes due to attenuated TGFβ signaling which interrupts hepatocyte differentiation. These data suggests that HS in hepatocytes prevents the differentiation of hepatocytes and has a negative impact on insulin sensitivity.

1-O-021 Oral Sessions

The ameliorating effect of molecular hydrogen on intestinal injury in high fat diet-loaded senescence-accelerated mouse

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【Subject】It is suggested that aging and excessive intake of fat may induce dysbiosis and intestinal inflammatory damage additively. Recent studies reported that molecular hydrogen has anti-inflammatory effect and antioxidant effect. Therefore, we analyzed the effects of molecular hydrogen on dysbiosis and intestinal injury induced by aging and excessive intake of fat.

【Method】Senescence-accelerated mouse prone-8 (SAMP8) were fed control diet or high fat diet (HFD) for 14 weeks, and then the each group was fed placebo jelly or hydrogen-rich jelly for 4 weeks. After the treatment, small intestinal tissues were harvested for morphological examination as well as organoid analysis. Moreover, we analyzed alterations of microbiota composition in cecal feces by 16S rRNA gene analysis of microbiota profiling.

【Result & Conclusion】The treatment with hydrogen-rich jelly prevented intestinal morphological damage and suppressed the decreases of CDX2 and BrdU expression in HFD-loaded SAMP8 mice. The treatment also increased the number of cultured organoids derived from small intestine of HFD-loaded SAMP8 mice. The 16S rRNA gene sequencing analysis suggested that the treatment decreased the abundance of Proteobacteria phylum in HFD-loaded SAMP8 mice. These findings suggest that the treatment with molecular hydrogen may modify microbiota composition and suppress intestinal injury and regeneration dysfunction.

Effects of Rikkunshi-To on the donepezil-induced gastrointestinal symptoms in mice

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Donepezil is used for treatment of Alzheimer's disease, but it is associated with increased risk for gastrointestinal (GI) symptoms, such as anorexia, nausea, and vomiting. Their insufficient control affects the ability to continue the donepezil therapy. Rikkunshi-To (RKT), a traditional herbal Japanese medicine, has been prescribed for patients with various GI symptoms because it improves the GI function via the potentiation of ghrelin signaling pathway. In this study, we investigated the effects of RKT on the prevention of donepezil-induced anorexia, nausea, and vomiting in mice and the involvement of ghrelin in its therapeutic effect. We have reported that donepezil (5mg/kg, i.p.) induced anorexia and pica, kaolin ingestion behavior, could be used to evaluate nausea and vomiting in mice fed the normal diet. Mice fed the diet supplemented with RKT (1%) did not show donepezil-induced anorexia and pica, and this therapeutic effect was antagonized by pretreatment with the ghrelin receptor antagonist. Donepezil significantly suppressed the intestinal motility in mice fed the normal diet; however, RKT recovered the motility delay. Furthermore, the ghrelin receptor antagonist reduced the effect of RKT to improve the intestinal motility. These findings suggest that RKT is a candidate for the treatment of donepezil induced anorexia, nausea, and vomiting in human patients, and that the enhancement of ghrelin signaling is involved in its therapeutic effect.

Doxorubicin impaired mitochondrial respiration lead to cause the lethal cardiotoxic damage

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Doxorubicin (Dox) is one of the practical anti-cancer agents against various tumor cell types. Unfortunately, the use of dox has been hampered due to the life-threatening cardiotoxic damage such as lethal cardiomyopathy it has caused. Mitochondria are double-membrane organelles that play a critical role in cellular homeostases, like ATP supply by oxidative phosphorylation. Many reports suggested that Dox impairs mitochondrial function that causes cardiomyopathy. However, the effect of Dox on oxidative phosphorylation has remained elusive. To confirm the effects of Dox on mitochondrial oxidative phosphorylation, we evaluated the effect of Dox on rat embryonic cardiomyoblast H9c2 cells by measuring oxygen consumption rate (OCR). Administration of 1 μM Dox caused significant decreases in the basal OCR. The addition of 1 μM Dox in the presence of oligomycin exhibited a significant increase of OCR compared to that without Dox, suggesting that Dox damaged regulation of the mitochondrial respiratory system. FCCP treatment with 1 μM Dox caused a significant reduction of the maximal respiratory. We also evaluated the extracellular acidification rate (ECAR). Administration of 1 μM Dox caused a significant increase or showed a tendency to increase in the basal ECAR and the presence of oligomycin, but not in the presence of FCCP. These results indicate that Dox impaired the mitochondrial respiration system and induced the metabolic shift of the cells in the presence of 1 μM of Dox occurred in the basal condition from oxidative phosphorylation to a glycolytic pathway to produce ATP.

Development of Supportive Care for Anticancer Drugs Based on Real World Data; Evaluation of the Therapeutic and Preventive Effects of Statins on Peripheral Neuropathy

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Chemotherapy-induced peripheral neuropathy (CIPN) is one of the adverse events associated with the anticancer drugs, however, almost available analgesic drugs lack efficacy against CIPN. Previously our results using medical database, FAERS, suggested that HMG-CoA reductase inhibitors (statins) have the potential to ameliorate oxaliplatin-induced peripheral neuropathy (OIPN). In this study, we elucidated the effect and mechanism of statins to OIPN model mice and PC12 cell. Three statins (simvastatin, atorvastatin, and rosuvastatin) could not show the therapeutic and preventive effects against oxaliplatin-induced cold allodynia. On the other hand, repeated orally administration of each statins ameliorate development of oxaliplatin-induced mechanical allodynia and significantly suppressed already established allodynia induced by oxaliplatin. A gene-related database revealed that the expression of glutathione S-transferase (GST) family members is regulated by statins. Decreased survival rate of PC12 cells by treatment of oxaliplatin was canceled cotreatment of each statin for 24 hours. Furthermore, cell protective effect of statin was disappeared transfection of gstmu1 siRNA into PC12 cells. These our results suggest that statins might be one of the novel supportive care, which have neuroprotective effect to OIPN.

Research and Development of Next Generation Zebrafish Developmental Toxicity Screening System

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The ICH and the PMDA published their guideline S5(R3) for the evaluation of reproductive toxicity of medicinal products, and has started to develop zebrafish developmental toxicity testing in Japan. In this study, we attempted to establish a zebrafish quality control protocol, which is the most important accuracy basis for the zebrafish developmental toxicity test. We first analyzed 12,906 fertilized eggs and found that 297 (2.3%) eggs died by 3 hours after fertilization. And 2,104 (16.3%) had morphological abnormalities by 3 hours after fertilization. By 24 hours after fertilization, 4,646 eggs (36.0%) were found to be dead. However, the survival rates from 1 to 5 days after fertilization were stable compared to the rapid decline in survival rate at 24 hours after fertilization. On the other hand, the results of time-lapse imaging from 3 hours to 5 days after fertilization showed that abnormal egg imaging at 3 hours after fertilization could predict death or morphological abnormalities up to 5 dpf. As a result, we report that it was possible to remove these low-quality fertilized eggs before the start of compound exposure (6 hpf) for developmental toxicity testing, thus reducing the frequency of false positives and enhancing the accuracy of zebrafish developmental toxicity screening.

Conducting online pharmacology role-playing on medical-students - consideration of pros and cons from the results of a two-year students questionnaire survey-

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Due to the coronavirus pandemic situation, the pharmacological role-playing was conducted by online in 2020 and 2021. We reported consideration of pros and cons of the online pharmacology role-playing from results of a two-year student questionnaire survey. Two hundred twenty five 3rd-grade medical students joined the role-playing at Dokkyo Medical University. Twenty-four students were assigned as physicians or patients and played the informed consent in two cases; hypertension accompanying diabetes and dementia with polypharmacy. The remaining students were observers. All students answered the questionnaire with a score of one to five, regarding the usefulness of study on disease and pharmacotherapy, understanding the patients' feeling, improvement of motivation to become a doctor, and change of attitude to studying. The percentage of students who scored five or four was 63 to 83% for the players and 71 to 80% for the observers. The frequent answers regarding necessary points of the study were "quality of the study" and "communication ability" and "perspective from the patients" for the players, and "communication ability", "quality of the study" and "perspective from the patients" for the observers in order of frequency. Most students described positive impression in the free entry field that was considered as pons. A description of the difficulty of adjusting the online settings was considered as cons. Online pharmacological role-playing may be a useful approach for the medical students to learn the pharmacotherapy and doctor-patient relationship.

Inflammatory responses by retinal astrocytes during pathogenesis of normal-tension glaucoma

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Glaucoma is the first cause of blindness in Japan, which is characterized by progressive degeneration of retinal ganglion cells (RGCs). Although an elevated intraocular pressure (IOP) is one of major risk factors, many Japanese glaucoma patients show normal level of IOP (i.e. normal tension glaucoma, NTG). We have recently discovered a novel NTG model (Astro-KO) mouse in which astrocytes lack the gene encoding ATP-binding cassette transporter A1 (ABCA1). The NTG mice at 3 months old (mo) showed no RGC damages but showed significant damages of RGCs and visual impairment at 12 mo. Although dysfunction of astrocytes was essential for NTG-like pathologies, molecular mechanisms remained unclear. To tackle this, we performed bulk and single-cell RNA-sequence of retina. We found that RGCs and retinal astrocytes up-regulate neuroinflammatory pathways including CXCR4 and CCR5 signaling. Immuonhistochemical analysis revealed that CXCL12 and CCL5, ligands for CXCR4 and CCR5, were up-regulated in retinal astrocytes of Astro-KO mice at 12 mo. CXCR4 and CCR5 in Astro-KO mice (12 mo) were expressed in the ganglion cell layer. Taken together, our data showed that lack of ABCA1 triggers neuroinflammation by astrocytes, which may cause RGC damages via CXCR4 and CCR5 activation.

Macroscopic imaging analysis of astrocytic Ca^{2+} activities in neurodegenerative disease model mouse

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Astrocyte is the predominant type of glial cell that attracts attention as a potential target of drug discovery and therapeutics for neurodegenerative diseases. Astrocytic Ca^{2+} signals are enhanced by neuronal hyperactivities and/or bioactive substances released from injured brain cells. Ca^{2+} signals can trigger Ca^{2+}-dependent processes including various gene expressions and secretion of neuro-protective/toxic molecules in astrocytes. Thus, analysis of astrocytic Ca^{2+} signals may provide clues for the regulation of neurodegenerative diseases. Because it remains elusive how the pathological conditions affect astrocytic Ca^{2+} activities, we are trying to establish a new method to analyze astrocytic Ca^{2+} activities in neurodegenerative diseases. We applied a drug-induced or genetical neurodegenerative disease model to a transgenic mouse line that expresses a genetically encoded Ca^{2+} sensor, YC-Nano50, in astrocytes. We found disorder-related changes in astrocytic Ca^{2+} activities from macroscopic Ca^{2+} imaging analysis in the cortical surface of these mice. This method may clarify astrocytic Ca^{2+} activities in brain pathology and contribute to the development of therapeutic strategies for neurodegenerative diseases.

1-O-029 Oral Sessions

The modulation of purine metabolisms by fibroblast growth factor 2 via several intracellular signaling pathways in cultured astrocytes

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Extracellular purines, including ATP and adenosine (ADO), are important neurotransmitter and neuromodulator in the central nervous system (CNS). Purine concentration is controlled by purine release and metabolisms. Astrocytes play important role in purine metabolisms in the CNS. In our previous study, we have shown that fibroblast growth factor 2 (FGF2) upregulates purine metabolic enzymes in rat spinal cord astrocytes. In this study, we investigated the effects of FGF2 on purine metabolisms in rat cortical astrocytes.

Cultured astrocytes from rat cerebral cortex were treated with FGF2. Enzymatic activity for purine metabolism was measured by incubation with extracellular solution containing ATP, AMP or ADO and measurement of those metabolites with HPLC. The expression levels of enzymes were measured by real-time PCR.

In cultured cortical astrocytes, FGF2 increased the mRNA and activity of ecto-5'-nucleotidase (CD73) and adenosine deaminase (ADA), and decreased those of ectonucleoside triphosphate diphosphohydrolase 2 (ENTPD2). An FGF receptor inhibitor, SU5402, inhibited the changes in the expression and activity of CD73, ADA and ENTPD2. U0126, a MEK inhibitor, and SP600125, a JNK inhibitor, inhibited the increase of CD73 and ADA, respectively. On the other hand, neither U0126 nor SP600125 inhibited the decrease of ENTPD2.

These results indicate that FGF2 modulates the expression and activity of CD73, ADA and ENTPD2 through FGF receptor. Furthermore, it is suggested that different intracellular signaling pathways are involved in modulation of each purine metabolic enzymes.

Effect of tumor releasing factor, cGAMP on gene expression and calcium response in astrocytes

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【Purpose】

Metastatic brain tumors emit various components, including cyclic 2'3'-GMP-AMP (cGAMP) to surrounding astrocytes, which may affect the tumor microenvironments. To identify the cGAMP targets, we analyzed transcriptome in cGAMP-incorporated astrocytes.

【Method】

cGAMP is encapsulated in lipid nanoparticles (ssPalm), then introduced into primary cultured astrocytes. After extracting the RNA, the fold change (FC) by cGAMP was investigated by microarray. Among the genes with high intrinsic expression levels, we searched for interacting factors with large FC in STRING's protein-protein interaction database. As an indicator of astrocyte responsiveness, changes in intracellular calcium concentration were observed by confocal time-lapse imaging.

【Result/Discussion】

As candidates of cGAMP targets, interferon-stimulated genes were determined, such as Viperin and Usp18. Cholecystokinin (CCK), which was reported to be involved in glutamate secretion, showed the largest FC(318-fold). Further, the intracellular calcium concentration increased when CCK was added to astrocytes. We have previously demonstrated that the introduction of cGAMP alters glutamine-glutamate metabolism in astrocytes. We continue to be interested in how these changes relate to each other and contribute to the malignant transformation of metastatic brain tumors.

Astrocytic control of microglial engulfment during postnatal development, which fates a lifelong cortical circuits

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Glial cells are vital for the modulation of synaptic connections and healthy brain development. They control the excitatory / inhibitory synaptic balance and assemble neural circuity by synaptic formation or elimination. We have recently revealed that astrocytes form excitatory synapses in the adult injured brain through mGluR5 signaling. However, astrocytic mGluR5 is, in health brain, expressed in the limited time-window of the postnatal developmental stage. Thus, we surveyed whether / how astrocytic mGluR5 destines the subsequent synaptic assembly using astrocyte-specific mGluR5 KO mice (cKO). Unexpectedly, the number of excitatory synapses did not alter much in cKO, instead, the number of inhibitory synapses decreased significantly in cKO throughout ages. Interestingly, microglia frequently engulfed inhibitory synaptic elements in cKO. Astrocytic mGluR5 expression was transient event in the critical period, however, behavioral dysfunction was observed even in adult cKO mice. Next, we surveyed the astrocytic molecule which regulates microglial engulfment. We focused on astrocytic IL7, which is decreased in cKO, as a candidate for such molecules. IL7 treatment decreased engulfment-related gene expression in microglia. Hence, we conclude that astrocytes organize inhibitory network in the critical period by modulating microglial phagocytic activity. It should be noted that although mGluR5 is only transiently expressed in astrocytes in the critical period, its function greatly affects the inhibitory neuronal networks throughout life.

Cardioprotective Effects of a Nonsteroidal Mineralocorticoid Receptor Blocker, Esaxerenone, in Dahl Salt-Sensitive Hypertensive Rats

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We investigated the effects of esaxerenone, a novel, nonsteroidal, and selective mineralocorticoid receptor blocker, on cardiac function in Dahl salt-sensitive (DSS) rats. We provided 6-week-old DSS rats a high-salt diet (HSD, 8% NaCl). Following six weeks of HSD feeding (establishment of cardiac hypertrophy), we divided the animals into the following two groups: HSD or HSD + esaxerenone (0.001%, *w/w*). In survival study, all HSD-fed animals died by 24 weeks of age, whereas the esaxerenone-treated HSD-fed animals showed significantly improved survival. We used the same protocol with a separate set of animals to evaluate the cardiac function by echocardiography after four weeks of treatment. The results showed that HSD-fed animals developed cardiac dysfunction as evidenced by reduced stroke volume, ejection fraction, and cardiac output. Importantly, esaxerenone treatment decreased the worsening of cardiac dysfunction concomitant with a significantly reduced level of systolic blood pressure. In addition, treatment with esaxerenone in HSD-fed DSS rats caused a reduced level of cardiac remodeling as well as fibrosis. Furthermore, inflammation and oxidative stress were significantly reduced. These data indicate that esaxerenone has the potential to mitigate cardiac dysfunction in salt-induced myocardial injury in rats.

Effect of low Na^+ solution on the spontaneous electrical activity and Ca^{2+} dynamics in guinea pig sinoatrial node.

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In the heart, the frequency of spontaneous electrical activity generated in the sinoatrial node (SAN) is determined by diastolic depolarization which is believed to be formed by ion channel-dependent membrane potential changes. It has also been proposed that intracellular Ca^{2+} may regulate spontaneous activity via electrogenic Na^+/Ca^{2+} exchanger (NCX); Ca^{2+} clock theory. In this study, we investigated whether NCX contributes to spontaneous activity in guinea pig SAN.

Immunofluorescence staining showed the expression of NCX on the cell membrane of the SAN cells.

To evaluate the role of NCX in spontaneous activity and Ca^{2+} dynamics, high-speed Ca^{2+} imaging was performed on SAN cells and spontaneous Ca^{2+} transients were observed. The inhibition of NCX activity by perfusion with low Na^+ solution resulted in an increase in the basal fluorescence intensity, but had little effect on the frequency of Ca^{2+} transients. Glass microelectrode recording of action potential revealed that low Na^+ solution has no effect on the diastolic depolarization. Furthermore, SEA0400, an inhibitor of NCX, did not affect the frequency of spontaneous activity and the diastolic depolarization.

These results suggest that, although NCX is expressed in guinea pig SAN cells and is responsible for Ca^{2+} efflux, its contribution to spontaneous activity is very small.

Roles of autophagy in angiotensin II-induced cardiac myocyte apoptosis

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[Background] Autophagy is a self-degradation system of intracellular organelles and found in failing heart. We investigated whether angiotensin II (ANG II) enhanced myocyte autophagy and the role of autophagy in ANG II-induced injury. [Methods and Results] Neonatal rat cardiac myocytes were treated with ANG II (1–100 nmol/L). ANG II dose dependently increased autophagy, assessed by microtubule-associated protein 1 light chain (LC) 3-II expression. It also enhanced the intracellular reactive oxygen species (ROS), detected by H2DCFDA staining. NADPH oxidase- and mitochondria-derived ROS production was increased by ANG II, while ANG II-induced autophagy was suppressed by inhibitors of these sources of ROS. ROS-producing mitochondria colocalized with lysosomes after ANG II stimulation. Myocyte apoptosis was observed using nuclear staining with DAPI. A 6-hour stimulation with ANG II did not affect myocyte apoptosis, while a co-treatment with 3-methyl-adenine (3MA), an autophagy inhibitor, increased apoptosis. A longer ANG II stimulation for 24 hours induced apoptosis, while the co-treatment with 3MA did not lead to further increase. [Conclusion] ANG II enhanced intracellular ROS production, leading to autophagy in myocytes. Autophagy was beneficial because it removed damaged mitochondria, which suppressed myocyte apoptosis in the early stages of the ANG II stimulation, while the longer ANG II stimulation itself induced apoptosis that was not effectively suppressed by autophagy.

2,5-Dimethylcelecoxib attenuates cardiac fibrosis caused by cryoinjury-induced myocardial infarction by suppressing the fibroblast-to-myofibroblast transformation via inhibition of TGF-b signaling pathway

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We previously reported the 2,5-dimethylcelecoxib (DM-C) attenuated cardiac remodeling in different types of cardiac hypertrophy model. However, it remains unclear whether DM-celecoxib attenuates fibroblast-to-myofibroblast transformation (FMT) which plays the key role in cardiac fibrosis after myocardial infarction (MI). Therefore, we investigated the effect of DM-C on FMT using cryoinjury induced myocardial infarction (CMI) mouse model and TGF-β1-stimulated cardiac fibroblasts. We found that DM-celecoxib attenuated deterioration of left ventricular ejection fraction after CMI by decreasing cardiac fibrosis. Analysis of the expression level of α-smooth muscle actin, a marker for myofibroblast, indicated that DM-celecoxib decreased FMT in cardiac injured site. In the cardiac fibroblasts, DM-celecoxib suppressed expression of α-SMA and phosphorylation levels of Smad 2/3 and GSK-3, indicating that DM-celecoxib suppressed α-SMA expression by inhibiting TGF-β signaling pathway via activation of GSK-3. These results suggested that DM-celecoxib attenuated cardiac fibrosis via suppressing fibroblast-to-myofibroblast transformation in injured site after CMI by suppressing TGF-β signaling pathway via activation of GSK-3. Thus, DM-celecoxib has a potential as a novel anti-fibrotic agent after MI in clinical setting.

TGFβ3 exacerbates myocardial remodeling after myocardial infarction.

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After myocardial infarction (MI), various kinds of cytokines are produced from pro- and/or anti- inflammatory cells that infiltrated into myocardium and contribute to scar formation and/or tissue repair. Among these cytokines, it is widely accepted that TGFβ family performs multiple functions, such as cell proliferation and fibrosis; however, it remains to be fully clarified whether there are functional differences among TGFβ1, 2, and 3. The aim of this study is to elucidate the pathophysiological significance of TGFβ3 after MI. MI was generated by coronary ligation. We measured the expression of TGFβ3 over time after MI by quantitative RT-PCR, and found that the expression of TGFβ3 mRNA peaked 7 days after MI (17.8±12.9 fold v.s non-MI). Quantitative RT-PCR and immunohistological staining showed that TGFβ3 was mainly expressed at the border region of infarction. To examine the effects of TGFβ3 on post-infarct remodeling, we administered TGFβ3 neutralizing antibody (TGFβ3 nAb) intravenously after MI. Echocardiographic analysis revealed that TGFβ3 nAb reduced cardiac dysfunction (fractional shortening: control IgG;29.8±7.3%, TGFβ3 nAb; 36.9±5.8%). In addition, Masson trichrome staining showed that neutralizing antibodies inhibited cardiac fibrosis after MI. TGFβ3 could promote adverse cardiac remodeling after MI as a novel therapeutic target.

1-SS-67 YIA

Structural analysis of the ABC transporter which transports long chain fatty acid CoA.

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Fatty acid coenzyme A (CoA), which is synthesized from fatty acids and CoA, plays an important role in lipid metabolism as it is used for beta oxidation and lipid biosynthesis. Beta oxidation, a metabolic pathway for degrading fatty acid CoA to extract acetyl CoA, occurs at mitochondria and peroxisomes. Beta oxidation in mitochondria plays a vital role in supplying acetyl CoA, FADH2, and NADH to TCA cycle, whereas in peroxisomes plays an important role in the degradation of lipids, including very long chain fatty acid CoA, which cannot be transported to mitochondria. ABCD1, ABCD2, and ABCD3, which belong to the ATP-binding cassette transporters (ABC transporters), transport long chain fatty acid acyl CoA from cytoplasm to peroxisome. ABCD1, 2, and 3 have a difference in the specificity of substrate. ABCD1 and 2 mainly transport saturated long chain fatty acid CoA, whereas ABCD3 transports unsaturated long chain fatty acid CoA and branched fatty acid CoA. Several structures have been reported about ABCD1, but the structure of ABCD3 has not yet been elucidated. We analyzed the structure of ABCD3 to obtain a detailed structural basis for its substrate specificity. As a result, we determined ATP bound ABCD3 structure reconstituted into lipid nanodisc which is similar to natural lipid environment than detergent at 4.5 Å. In the near future, we will acquire the structure of ABCD3 bound to its substrate and will elucidate the molecular basis of substrate specificity among ABCD family, providing insights into diseases caused by abnormal lipid metabolism.

1-YIA-01 YIA

Interactions of active zone proteins with Munc13-1 that contribute to the formation of synaptic vesicle release sites

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Synaptic vesicle exocytosis is regulated by several proteins localized at the active zone of presynaptic terminals. Munc13-1 is a multi-domain active zone protein which redundantly interacts with other active zone proteins. We previously found that Munc13-1 forms supramolecular nanoassemblies that correspond to the sites of synaptic vesicle exocytosis. Here, we aimed to clarify a more detailed molecular mechanism of Munc13-1 in the formation of synaptic vesicle release sites by intervening in the interactions between Munc13-1 and other active zone proteins. For this purpose, we acquired a series of isolated functional domains which directly bind to Munc13-1, from active zone proteins including RIM, CAST, RIM-BP, and Piccolo. Among these domains, we found that the expression of Zn^{2+} finger domain of RIM (RIM-ZF), which binds to C_2A domain of Munc13-1, caused a significant decrease in neurotransmitter release, although a previous study showed that the RIM-ZF partially rescues suppressed neurotransmitter release in RIM knock-out neurons. Furthermore, quantitative immunocytochemical analysis with super-resolution microscopy imaging revealed that the expression of RIM-ZF selectively reduced the amounts of Munc13-1 molecules at the active zones. Thus, our results suggest that a direct interaction of RIM-ZF with Munc13-1 itself is incomplete for the appropriate formation of synaptic vesicle release sites, and that cross-linkage of RIM-ZF to the other domains of RIM is necessary for the precise positioning of Munc13-1 at the active zone.

1-YIA-02 YIA

Analysis of the effect on TARPγ-8-PSD95 coupling on TARPγ-8 dynamics

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AMPA receptors (AMPAR) auxiliary subunit TARP γ-8 is a critical molecule for recruiting AMPAR to hippocampal excitatory synapses. Although biochemical interaction of TARP γ-8 with PSD95 has been reported, it is not fully understood how the TARP γ-8-PSD95 interaction contributes to the distribution of TARP γ-8. Here we aimed to reveal the distribution of TARP γ-8 in neurons and to examine the effect of TARP γ-8-PSD95 coupling on spatiotemporal dynamics of TARP γ-8. First, we confirmed that TARP γ-8 was accumulated at the synapse in cultured neurons by immunostaining. Then we examined the effect of PSD95 on the intracellular distribution of TARP γ-8 by heterologous expression of fluorescent protein tagged constructs in COS7 cells. TARP γ-8 and PSD95 formed molecular clusters with the typical size of ~1 μm, while TARP γ-8 solely expressed in cells showed diffuse pattern. Furthermore, we performed single-particle tracking of TARP γ-8, revealing that the TARP γ-8 molecules within the clusters were in an immobile state with the diffusion coefficient (D) of less than 0.01 $\mu m^2/s$, while the TARP γ-8 molecules outside the clusters were highly mobile with D of ~0.05 $\mu m^2/s$. Our results suggest that PSD95 assemblies regulate the dynamics of TARP γ-8, and that PSD95-TARP γ-8 coupling plays a functional role in recruiting AMPAR to the synaptic membrane.

1-YIA-03 YIA

Potential role of G protein-coupled receptor 3 in the axonal regeneration after optic nerve injury in mice

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G protein-coupled receptor 3 (GPR3) is highly expressed in various neurons, and unique in its ability to constitutively activate the Gαs protein without the addition of ligands, which elevates the basal level of intracellular cAMP. In our earlier study, we clarified that GPR3 play a role in both neurite outgrowth and neuronal survival in developing cerebellar granular neurons. However, the potential role of GPR3 in the axon regeneration still remains unclear. Herein, we investigated the possible involvement of GPR3 in axonal regeneration after optic nerve injury in mice. GPR3 expression was observed in the retinal ganglion cells (RGCs). GPR3 expression was increasingly expressed during development in the mouse primary retinal ganglion neurons (RGNs). When GPR3 expression was suppressed by siRNA, both neurite outgrowth and survival in RGNs were significantly inhibited. In the pressure-induced retinal ischemia model, the number of RGCs and the inner plexiform layer thickness were significantly reduced in the GPR3 knockout mice compared to those in wild-type mice 7 days after the induction of transient retinal ischemia. In addition, regenerating axon was significantly increased 4 weeks after optic nerve crush (ONC) when GPR3 was overexpressed in RGCs using adeno-associated virus. Furthermore, axonal regeneration was dramatically augmented when Zymosan was inoculated in the GPR3-overexpressed retina. These results suggest that GPR3 play a potential role in accelerating axonal regeneration after ONC in mouse.

1-YIA-04 YIA

L-DOPA receptor, GPR143, is involved in methylphenidate -induced locomotion in mice

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Methylphenidate(MPH) is widely known as a drug for attention deficit hyperactivity disorder as well as an addictive drug, and its main pharmacological action is thought to elevates extracellular dopamine(DA) level by inhibiting presynaptic DA transporter. L-3.4-dihydroxyphenylalanine(DOPA) has been believed as a precursor of DA. We proposed that DOPA itself acts as neurotransmitter. We previously demonstrated that nicotine, and methamphetamine (METH) that shares pharmacological properties with MPH, increased not only extracellular DA level but also DOPA level in the nucleus accumbens of mice, while cocaine increased the DA but suppressed the DOPA level. Recently we identified G protein coupled receptor GPR143, a gene product of ocular albinism-1, as a receptor for DOPA. We showed that GPR143 was involved in the pharmacological actions of nicotine (Masukawa et al, 2020). In this study, we compared actions of MPH between wild-type (Wt) and *Gpr143* gene-deficient (*Gpr143*$^{-/y}$) mice. MPH-treated mice showed an increase in locomotor activity compared to the saline-treated group. This effect was attenuated in *Gpr143*$^{-/y}$ mice. Cocaine-treated mice also showed an increase in locomotor activity in Wt and *Gpr143*$^{-/y}$ mice compared to corresponding controls, but there was no difference in this effect between Wt and *Gpr143*$^{-/y}$ mice. These results suggest that GPR143 is involved in the pharmacological action of MPH.

1-YIA-05 YIA

Molecular characterization of the presubiculum of mouse during development

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The presubiculum is a subarea of the parahippocampal region and plays a crucial role in spatial navigation. In particular, excitatory neurons in the presubiculum selectively fire when the head of the animal points to a specific direction. This head-direction selectivity emerges before eye opening in rodents and is maintained in adulthood through synaptic interactions between excitatory and inhibitory neurons. Although the developmental spatial representation of the presubiculum has been electrophysiologically studied, the histological characteristics of the developing presubiculum are poorly understood.

In this study, we anatomically identified the presubiculum using an anterograde tracer injected into the anterior thalamic nucleus and found that the superficial layers of the presubiculum could be delimited by the expression of vesicular glutamate transporter 2 (VGluT2). We also immunostained the brain slices of mice aged from neonates to adults using antibodies against parvalbumin (PV) and somatostatin (SOM) and found that in the presubicular superficial layers, PV-positive interneurons progressively increased in number during development, whereas the number of SOM-positive neurons exhibited no specific trend.

1-YIA-06 YIA

GPR143, a L-DOPA receptor, induced migration and proliferation of vascular smooth muscle cells is involved in monocrotaline-induced pulmonary hypertension in rats

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We previously demonstrated that L-DOPA modulated the vascular α1-adrenergic receptor through GPR143, a G-protein coupled receptor, and sensitized vasomotor tone. In this study, we examined a possible role of GPR143 in the pathogeneisis of pulmonary hypertension (PH). In isolated pulmonary arteries, L-DOPA (1 μM) augmented contractile response to phenylephrine, an α1 adrenergic receptor agonist. We generated GPR143 gene-deficient (*Gpr143*$^{-/y}$) rats and comparatively studied the effect of L-DOPA. L-DOPA did not modify phenylephrine-induced response in the pulmonary arteries of *Gpr143*$^{-/y}$ rats, thereby indicating that the action of L-DOPA was mediated by GPR143. We next established monocrotaline (MCT, 60 mg/kg) -induced PH model in wild type (WT) and *Gpr143*$^{-/y}$ rats. One month after injection subcutaneously with MCT , the right ventricular systolic pressure (RVSP) was attenuated in *Gpr143*$^{-/y}$ rats as compared to the WT rats (49.7 +/- 1.1 mmHg and 41.4 +/- 1.4 mmHg in WT and *Gpr143*$^{-/y}$, $p<0.05$, N=5). Coordinately, the right ventricle to body weight (RV/BW) (5.4 +/- 0.2 × 10^{-4} and 4.7 +/- 0.1 × 10^{-4} in WT and *Gpr143*$^{-/y}$, $p<0.01$, N=12) was also reduced in *Gpr143*$^{-/y}$ rats compared to the WT rats. Furthermore, in primary cultures of pulmonary artery smooth muscle cells (PASMCs), the proliferative and migratory capacity of *Gpr143*$^{-/y}$ PASMCs after phenylephrine treatment was reduced compared to *Gpr143*-WT PASMCs. We here provide evidence that GPR143 may be involved in MCT-induced PH in rats by affecting the proliferative and migratory capacity of PASMCs.

1-YIA-07 YIA

Aldosterone enhances stability of atrial fibrillation in the rat model of chronic volume overload

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Purpose: Chronic volume overload to the heart has been suggested to generate arrhythmic substrates in the atria. We investigated whether aldosterone modifies inducibility of atrial fibrillation (AF) in rats with aorto-venocaval shunt (AVS).

Methods and Results: To elevate plasma aldosterone concentrations by 1.5 to 4.5-fold, aldosterone was intraperitoneally administered at 0.5, 1.0 or 1.5 μg/h for 4 weeks using an osmotic minipump that was implanted at the operation of AVS. Four weeks after the surgery, the duration of AF induced by burst pacing was 13±3 s in control AVS rats, whereas those in AVS rats receiving aldosterone at 0.5, 1.0 and 1.5 μg/h were 51±15, 75±18 and 99±34 s, respectively. The spontaneous premature atrial contraction often appeared in AVS rats receiving aldosterone, which was rarely observed in control AVS rats. There were no significant differences in the atrial effective refractory period, P-wave duration or atrial tissue weight among the all animal groups. Concomitant administration of spironolactone (100 mg/kg/day, p.o.) prevented the aldosterone-promoted AF.

Conclusions: Aldosterone enhanced stability of AF through mineralocorticoid receptors in the rat model of chronic volume overload. The results may partly reflect an increased risk of AF as observed in patients with primary aldosteronism.

1-YIA-08 YIA

Electropharmacological characterization of anti-atrial fibrillatory drug vernakalant using the isoflurane-anesthetized dogs

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Introduction: We characterized *in vivo* electropharmacological profile of anti-atrial fibrillatory drug vernakalant.
Methods: Vernakalant was intravenously administered in doses of 0.3 and 3 mg/kg/10 min to the isoflurane-anesthetized beagle dogs (n=5). These results were compared with those of other anti-atrial fibrillatory drugs; ranolazine, dronedarone, amiodarone, bepridil and *dl*-sotalol.
Results: Vernakalant suppressed the sinus automaticity, ventricular contractility, and atrioventricular nodal and intraventricular conduction, whereas it increased the total peripheral vascular resistance, preload to the left ventricle and mean blood pressure. Meanwhile, vernakalant delayed the repolarization in a reverse frequency-dependent manner; and the prolongation of early/late repolarization was 43 ms/12 ms when QT-interval prolongation was the greatest, whereas it prolonged the atrial/ventricular effective refractory period by 32 ms/44 ms, respectively.
Conclusion: The atrial effective refractory period prolongation by vernakalant was intermediate among the drugs, but its atrial selectivity was the lowest. Prolongation of the early repolarization by vernakalant was the greatest, whereas that of the late repolarization was in the middle, indicating greater risk for intracellular Ca^{2+} overload-based ventricular arrhythmias.

1-YIA-09 YIA

Significant role of L-type Ca^{2+} channels for arrhythmic trigger of drug-induced long QT syndrome assessed in the acute atrioventricular block rabbit

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A class III antiarrhythmic drug nifekalant has been demonstrated to prolong the QT interval with a lower risk of torsade de pointes (TdP) than dofetilide. We pharmacologically analyzed differences of proarrhythmic mechanisms between the two drugs using a TdP model of acute atrioventricular block rabbits under the monitoring of the monophasic action potential (MAP) of the right ventricle. Intravenous administration of dofetilide (0.025 mg/kg) and nifekalant (0.3 mg/kg) prolonged the MAP duration in a similar extent, which were accompanied with an induction of early afterdepolarization (EAD) in 4 out of 5 animals and all animals, respectively. Meanwhile, TdP appeared in 4 and 1 out of 5 animals after the administration of dofetilide and nifekalant, respectively. Nifekalant-induced EAD and TdP were effectively inhibited by both pretreatment with a L-type Ca^{2+} channel inhibitor verapamil and a Na^{+}/Ca^{2+} exchanger inhibitor SEA0400. On the other hand, dofetilide-induced EAD and TdP, which could be similarly suppressed by verapamil, were hardly affected by SEA0400, suggesting the less dependency of Na^{+}/Ca^{2+} exchanger on induction of dofetilide-induced EAD and TdP. Thus, the results imply a significant role of L-type Ca^{2+} channels for generation of arrhythmic trigger of drug-induced long QT syndrome.

1-YIA-10 YIA

Effect of TMEM182, a novel membrane protein, on muscle differentiation

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This study examines the effects of transmembrane protein 182 (TMEM182), which is highly expressed specifically in muscle tissue, on muscle differentiation. Although it is known that TMEM182 is involved in muscle differentiation, the detail is not fully elucidated. Therefore, we generated mouse skeletal myoblast cell line, C2C12, overexpressing TMEM182 in a doxycycline (DOX) -inducible manner. The results showed that the DOX-treated C2C12 inhibited differentiation into myotubular cells. To determine the mechanism, we are now searching for TMEM182 binding proteins by mass spectrometry. Because iPS cells do not express TMEM182 without differentiation, we are comparing undifferenciated iPS cells with the cells overexpressing TMEM182. We are also trying to investigate the effect of TMEM182 on myocardial differentiation using iPS cell-derived cardiomyocytes (iPS-CM) overexpressing TMEM182. As a result, the expressions of Nkx2.5, a cardiac progenitor cell marker, and TNNT2, a cardiomyocyte marker, were both decreased in iPS-CM overexpressing TMEM182 compared to control iPS-CM. These data suggest that TMEM182 regulates the differentiation of myoblasts into myotubes not only in skeletal muscle but also in cardiac muscle.

1-YIA-11 YIA

Chronic social stress alters synaptic central metabolism for depression

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Chronic social stress induces emotional and cognitive disturbances and is a risk for mental illness. Reduced neuronal activity in the medial prefrontal cortex (mPFC) underlies these behavioral abnormalities. However, the subcellular origin and process of this neuronal change remain elusive. Here we examined ultrastructural and multi-omics changes in the mPFC with social stress in mice. Social stress caused the loss of dendritic branches with morphological alterations of mitochondria and induced synaptic shrinkage selectively at mitochondria-containing synapses. Social stress deteriorated mitochondrial functions at synapses with altered mitochondrial proteome and central metabolism in the mPFC. Pharmacological manipulation targeting mitochondria attenuated the synaptic shrinkage and depression-related behaviors. These findings show that chronic social stress alters the central metabolism at mPFC synapses, leading to neuronal pathology and depression-related behaviors.

1-YIA-12 YIA

Involvement of increased kynurenic acid through downregulation of kynurenine-3-monooxygenase in depressive-like behavior and dysregulation of hypothalamic-pituitary-adrenal axis induced by chronic unpredictable mild stress

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Chronic stress contributes to the pathogenesis of major depressive disorder (MDD). In the kynurenine pathway (KP), kynurenine is metabolized to 3-hydroxykynurenine (3-HK) by kynurenine 3-monooxygenase (KMO) and to kynurenic acid (KA) by kynurenine aminotransferase. KP alternation has been reported to be associated with the pathogenesis of MDD. We investigated the involvement of KP in the depressive-like behavior induced by chronic unpredictable mild stress (CUMS). Mice were randomly exposed to 9 kinds of mild stressors for 4 weeks. Corticosterone level in the serum and corticotropin-releasing hormone (CRH) mRNA level in the hypothalamus (HT) elevated immediately after CUMS. Further, KMO mRNA level was decreased, but KA content was increased in the prefrontal cortex (PFC). Because KA is α7 nicotinic acetylcholine receptor (α7nAChR) antagonist, we investigated the effects of nicotine (Nic) and galantamine (Gal : α7nAChR agonist) on the depressive-like behavior and dysregulation of HPA axis induced by CUMS. When Nic and Gal were administrated before exposure to each stressor during CUMS, they attenuated CUMS-induced decreased sociability. Although Nic failed to inhibit elevated corticosterone level in the serum immediately after CUMS, but suppressed that sustained elevation 1 week after CUMS. Alternation of KP from 3-HK to KA through downregulation of KMO may be involved in the depressive-like behavior and the sustained elevation of serum corticosterone 1 week after CUMS.

1-YIA-13 YIA

A selective delta opioid receptor agonist, KNT-127, exerts an antidepressant-like effect and facilitates neuronal excitability in the mouse infralimbic prefrontal cortex *via* PI3K-mTOR signaling

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Growing evidence demonstrates that the delta opioid receptor (DOP) is an attractive candidate for novel antidepressants with the potential to exhibit rapid action with few adverse effects. However, the underlying detailed functional mechanism remains elusive. We previously reported that the selective DOP agonist, KNT-127, produced robust antidepressant-like effects in the forced swimming test (FST) in mice. Thus, we attempted to identify the cellular mechanism underlying this effect. As a result, the selective mTOR inhibitor, rapamycin, and the PI3K inhibitor, LY294002, blocked the antidepressant-like effects of KNT-127 in the FST. KNT-127 promoted the phosphorylation of mTOR signal-related proteins, Akt and p70S6K, in the medial prefrontal cortex in the protein immunoblotting assay. The bilateral microinfusion of KNT-127 into the infralimbic cortex (IL-PFC) reduced immobility in the FST. Furthermore, whole-cell voltage-clamp recordings revealed that the frequency of mEPSCs in the IL-PFC increased and that of mIPSCs decreased with the bath application of KNT-127, which was blocked by pretreatment with rapamycin. Taken together, our results suggest that KNT-127 directly activates neuronal excitability in the mouse IL-PFC through PI3K-Akt-mTOR-p70S6K signaling pathway to exert antidepressant-like actions. These results could indicate the first steps in elucidating the complete mechanical functions of DOPs as a potential candidate target for novel antidepressants.

1-YIA-14 YIA

Hemispheric asymmetry in chronic stress-induced microglial responses

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Chronic stress causes emotional disturbances and is a risk factor for mental illnesses such as depression. It was reported that chronic social stress activates microglia in the medial prefrontal cortex (mPFC) through innate immune receptors TLR2/4, leading to depressive-like behavior. Since lipopolysaccharide, a TLR4 ligand, differently activates microglia isolated from left and right hemispheres, we analyzed chronic social stress-induced gene expression changes in mPFC microglia of each hemisphere using laser microdissection microscopy combined with RNA-seq. Given individual variability in stress susceptibility, we categorized the stressed mice into susceptible and resilient mice based on the level of social avoidance. We identified genes that increased or decreased in expression in mPFC microglia with chronic stress. Many of these genes responded to stress differently between left and right hemispheres and between susceptible and resilient mice. Notably, the genes altered in expression only in resilient mice showed hemispheric asymmetry in their stress responses. These findings demonstrate hemispheric asymmetry of chronic stress-induced microglial responses and suggest its relevance to stress resilience.

1-YIA-15 YIA

Downregulation of astrocytic connexin43 potentiates the antidepressant-induced brain-derived neurotrophic factor expression

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Connexin43 (Cx43) is highly expressed in astrocytes (AS), and its expression is reduced in the prefrontal cortex of depressive patients. It showed that some antidepressants increase the expression of neurotrophic factors like a brain-derived neurotrophic factor (BDNF) mediated by lysophosphatidic acid (LPA) receptors. The current study examined whether decrease in Cx43 expression affect their expression. 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. Knockdown of Cx43 increased the amitriptyline (AMI)-induced BDNF expression in cultured AS. This enhancement was significantly suppressed by blockade of LPA1/3 receptors, Src tyrosine kinase, or extracellular signal-regulated kinase (ERK). The current study revealed that reduced Cx43 expression potentiates the AMI-induced BDNF expression in cortical AS. In addition, LPA1/3-mediated Src-ERK signaling might be essential in the potentiation of AMI-induced BDNF expression in Cx43-downregulated AS. These results indicate the downregulation of Cx43 observed in the depressive patients might contribute to therapeutic effect of antidepressants.

1-YIA-16 YIA

The role of progranulin on cardiac remodeling after myocardial infarction

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Ischemic heart disease, represented by acute myocardial infarction (AMI), is the leading cause of death in the world. Although the survival rate after AMI has improved with the development of reperfusion therapy, there are still many patients with poor prognoses progressing to heart failure after AMI. Thus, it is essential to understand the pathogenesis after MI and explore a novel therapeutic target. Progranulin is a growth factor associated with wound healing and inflammation. We previously reported that administration of progranulin had the protective effects on cardiac injury after MI in animal models. However, the role of endogenous progranulin on cardiac remodeling after MI is still unelucidated. In this study, we evaluated the role of progranulin in the pathophysiology post-MI using *in vivo* MI model mice. The expression level and localization of progranulin in the heart of MI model mice were investigated using Western blotting and immunostaining. We also analyzed the cardiac remodeling and survival rate after MI in progranulin KO and WT mice. Progranulin expression significantly increased in the whole heart 3 and 7 days after MI. The localization of progranulin was observed at the infarct border area. Fibrosis area and heart weight/body weight ratio significantly increased in progranulin KO mice compared with WT mice after MI. The survival rate was significantly reduced in progranulin KO mice compared with WT mice. Thus, it is suggested that progranulin has a crucial role in the protection from heart failure after MI.

1-YIA-17 YIA

CaMKII inhibition prevent Doxorubicin-induced mitochondrial dysfunction, independent from Drp1 and MCU.

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Title: CaMKII inhibition prevent Doxorubicin-induced mitochondrial dysfunction, independent from Drp1 and MCU.

Background: Doxorubicin (Dox), an anticancer drug, causes mitochondrial dysfunction by reducing mitochondrial membrane potential, which leads to cardiotoxicity. Although Dox activates calmodulin kinase II (CaMKII), it is unclear whether and how CaMKII is involved in impaired MMP by Dox. In addition, Dox activates Drp1 and MCU by phosphorylation. However, their roles in mitochondrial dysfunction by Dox is remained to be determined.

Method: To clarify the roles of CaMKII in mitochondrial dysfunction by Dox in H9C2 cells, we tested inhibitory effects of CaMKII, Drp1, and MCU on impaired MMP by Dox with fluorescence dye, JC-1. Furthermore, we examined inhibitory effects of CaMKII and Drp1 on activated mitophagy by Dox with fluorescence dye, Mtphagy, and those of CaMKII and MCU on increased levels of mitochondrial Ca2+ content by Dox with fluorescence dye, Rhod2-AM.

Result: Dox exposure significantly reduced MMP, increased the levels of mitochondrial calcium content and number of the cells with mitophagy compared with control ($p<0.05$ in all). Whereas CaMKII inhibition significantly reversed these events caused by Dox ($p<0.05$), the inhibition of MCU and Drp1 showed no reversal effects on impaired MMP by Dox.

Conclusion: This study suggests that CaMKII is involved in mitochondrial dysfunction via impairment of MMP in Dox-induced cardiotoxicity, independent from Drp1 and MCU, suggesting that excessive mitophagy and increased mitochondrial calcium content are not the mechanisms. Further study is warranted to clarify a direct target of CaMKII to impair MMP.

1-YIA-18 YIA

Iron derived from ferritinophagy induces cardiomyocyte death and heart failure in mice

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Heart failure is a major public health problem, and abnormal iron metabolism is common in patients with heart failure. Although iron is necessary for metabolic homeostasis, it induces a programmed iron-dependent form of necrosis, ferroptosis. Iron release from ferritin storage is through nuclear receptor coactivator 4 (NCOA4)-mediated autophagic degradation, known as ferritinophagy. However, the role of ferritinophagy and ferroptosis in the stressed heart remains unclear. To examine the role of ferritinophagy in cardiomyocytes, cardiomyocyte-specific NCOA4-deficient mice were generated. The mice were subjected to pressure overload by means of transverse aortic constriction to induce heart failure. Deletion of *Ncoa4* in mouse hearts reduced left ventricular chamber size and improved cardiac function along with the attenuation of the upregulation of ferritinophagy-mediated ferritin degradation 4 weeks after pressure overload. Free ferrous iron overload and increased lipid peroxidation were suppressed in NCOA4-deficient hearts. A potent inhibitor of lipid peroxidation, ferrostatin-1, significantly mitigated the development of pressure overload-induced dilated cardiomyopathy in wild-type mice. Thus, the activation of ferritinophagy results in the development of heart failure, whereas inhibition of this process protects the heart against hemodynamic stress.

1-YIA-19 YIA

Endogenous YAP activity potentiates GSK3 inhibitors-induced proliferation of cardiomyocytes.

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【Background】

Mammalian cardiomyocytes (CM) largely cease to proliferate after birth. So far, several pathways, including activation of yes-associated protein (YAP) signaling and inhibition of glycogen synthase kinase (GSK)-3 signaling (i.e., activation of β-catenin), have been identified as candidate pathways that promote CM proliferation. The aim of this study is to clarify the crosstalk between these two signaling pathways.

【Methods/Results】

Consistent with previous reports, immunofluorescence (IF) with cell cycle marker revealed GSK-3 inhibitors (GSK3I) increased proliferation of neonatal rat CM (NRCM) and human iPS-derived CM (hiPSCM) under sparse culture condition. IF also revealed YAP is endogenously activated in NRCM and hiPSCM. YAP knock-down or pharmacological inhibition of YAP reduced GSK3I-induced CM proliferation without suppressing the β-catenin activation, suggesting endogenous activity of YAP potentiated the proliferative effects of GSK3I. Under dense culture condition of hiPSCM, GSK3I alone could not induce the CM proliferation; however, YAP activation by knocking down α-catenin restored their proliferative effects.

【Conclusion】

The activation of YAP potentiates GSK3I-induced proliferation of CM, proposing a novel strategy for the CM amplification.

1-YIA-20 YIA

The Interaction of the p300 with the BRG1 Increases the Acetylation levels of the H3K122 in Heart Failure

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Background: Epigenetic regulatory mechanisms such as histone post-translational modifications are involved in the heart failure (HF). Although the acetylation of tail domains, such as H3K9, has been extensively studied, that of H3K122, the globular domain, has received much less attention. The H3K122 acetylation directly activates transcription by destabilizing histone-DNA binding. However, the mechanism of these domains acetylation in the development of HF remains unknown.

Methods and Results: Cultured cardiomyocytes prepared from neonatal rats were treated with phenylephrine (PE). PE increased the acetylation of H3K9 and H3K122. The acetylation of H3K9 and H3K122 on the promoters of BNP and b-MHC, which are hypertrophic reaction genes, was increased in cardiomyocyte hypertrophy. In Dahl-salt sensitive rats, a heart failure model, *in vivo* ChIP assays revealed that the acetylation of H3K9 on the promoters of BNP and b-MHC was increased in left ventricular hypertrophy (LVH), while that of H3K122 was increased in HF. On the other hand, there was no difference in the amount of transcriptional coactivator p300 recruitment in LVH and HF. Interestingly, IP -WB showed that binding of p300 with BRG1, a key component of the SWI/SNF complex, was enhanced in HF. The recruitment of BRG1 was increased in HF compared to LVH. Moreover, PFI-3, a BRG1 inhibitor, suppressed PE-induced increases in the acetylation of H3K122 in cultured cardiomyocytes.

Conclusion: This study shows that the acetylation of H3K122 is enhanced via the interaction of p300 with BRG1 in heart failure.

1-YIA-21 YIA

Novel prevention target of peritoneal deterioration caused by peritoneal dialysis

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Background: Artificial dialysis is divided into peritoneal dialysis (PD) and hemodialysis. Although PD provides higher quality of life than hemodialysis, few patients choose PD because of its inevitable dangerous. Long term PD evokes peritoneal deterioration which in turn triggers fibrosis and injured intestinal transit. It is necessary to discover the novel prevention target of peritoneal deterioration, but there were few reports because of the lack of clinical mouse model.

Methods: Peritoneal deterioration model mice were produced by methylglyoxal (MGO). After producing the models, the thickness of fibrotic layer and the capacity of intestinal transit were detected. The mRNA expression and protein level of fibrosis-promoting factors in peritoneal cavity were also measured.

Results: Peritoneum fibrosis and intestinal transit were aggravated with MGO treatment in time-dependent manner. Moreover, the mRNA expression of factor X was notably up-regulated in peritoneal cells. Protein level of factor X in peritoneal lavage fluid was also up-regulated in time dependent manner.

Conclusion: In this study, we confirmed that MGO peritoneal deterioration model showed the pathology seen in clinical site. Moreover, we revealed that the production of factor X was up-regulated in accordance with the severity of pathology. Therefore, factor X might be the new prevention target of peritoneal deterioration caused by PD.

1-YIA-22 YIA

Efficacy of Keap1-Nrf2 protein-protein interaction inhibitor for glomerulosclerosis in mouse model of chronic kidney disease

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Bardoxolone methyl is an electrophilic agent that induces Nrf2 activation by irreversibly and covalently binding to the cysteine residue of Keap1. Ongoing clinical trials of Bardoxolone methyl show promising effects for patients with chronic kidney disease (CKD). However, irreversible Keap1 inhibitors such as Bardoxolone methyl may covalently bind to other proteins in a non-specific manner and induce side effects due to off-target activities. In this study, we developed a reversible Keap1 inhibitor UBE-1099, which highly selectively and non-covalently inhibits Keap1-Nrf2 protein-protein interaction (PPI) and induces Nrf2 activation. We evaluated its efficacy on glomerulosclerosis in mouse model of CKD (Alport syndrome: Col4a5-G5X). Similar to Bardoxolone methyl, UBE-1099 transiently increased proteinuria and reduced plasma creatinine in CKD model mice. Importantly, UBE-1099 improved the glomerulosclerosis, renal inflammation and fibrosis, and prolonged the lifespan of CKD model mice. Moreover, transcriptome analysis in glomerulus showed that UBE-1099 induced the expression of genes associated with cell cycle and cytoskeleton, which may explain its unique mechanism of improvement such as glomerular morphological change. Thus, our results firstly revealed the efficacy of Keap1-Nrf2 PPI inhibitor for glomerulosclerosis and CKD.

1-YIA-23 YIA

Increased OASIS in podocytes contributes to the disruption of kidney homeostasis

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[Background]
Podocytes, a component of glomerular filtration barrier, are damaged under various stresses in kidney diseases. Previously, we revealed that a transcription factor old astrocyte specifically induced substance (OASIS) in kidney myofibroblasts promoted kidney fibrosis. However, the role of OASIS in podocytes remains unclear.
[Methods/Results]
LPS treatment increased OASIS expression in podocytes. To examine the roles of OASIS in podocytes, we generated podocyte-specific *OASIS* knockout (cKO) mice. Podocyte-specific *OASIS* deletion suppressed LPS-increased serum creatinine level, but did not influence albuminuria and podocyte injury. Interestingly, on the other hand, OASIS cKO mice were protected from LPS-mediated tubular injury. Microarray analysis using OASIS-overexpressed podocytes revealed that PRKCI was negatively regulated by OASIS in podocytes. We also found that recombinant PRKCI suppressed LPS-induced tubular injury in HK-2 cells in a dose-dependent manner. Finally, we established podocyte-restricted OASIS overexpressing transgenic mice and revealed that OASIS overexpression in podocytes caused tubular injury and kidney fibrosis, concomitant with severe albuminuria and podocyte foot process effacement.
[Conclusion]
Upregulation of OASIS in podocytes disturbs kidney homeostasis, leading to renal dysfunction.

1-YIA-24 YIA

Stathmin participates in antiproliferative effects of eribulin in leiomyosarcoma cells

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Uterine leiomyosarcoma is one of the aggressive malignancies with a poor clinical outcome. Stathmin, a microtubule-destabilizing protein, has been reported to be overexpressed in many malignant tumors including uterine leiomyosarcoma. The activity of stathmin in microtubule destabilization is regulated by the protein phosphorylation. Eribulin is a microtubule-targeting agent which has been approved for treatment of the patients with inoperable or recurrent breast cancer and soft tissue tumors such as uterine leiomyosarcoma. We explored the role of stathmin in the antiproliferative effect of eribulin in leiomyosarcoma cell line (SKN). Eribulin stimulated stathmin phosphorylation and decreased stathmin protein expression in SKN. Although neither inhibitors of protein kinase A nor CaMKII affected the eribulin-induced stathmin phosphorylation, a protein phosphatase 2A (PP2A) activator FTY720 attenuated the phosphorylation. In addition, eribulin reduced the levels of PP2A A and C subunits. Notably, stathmin knockdown decreased the inhibitory effects of eribulin on cell viability, and stathmin overexpression potentiated the efficacy. Stathmin expression was markedly downregulated in eribulin resistant SKN lines we established. These results suggest the significance of stathmin dynamics in antiproliferative activity of eribulin in uterine leiomyosarcoma.

1-YIA-25 YIA

Effect of filtrated bone marrow derived mesenchymal stem cell lysate on erectile function in erectile dysfunction model rat with cavernous nerve injury

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[Objective] Radical prostatectomy is involved with cavernous nerve (CN) damage which often cause neurogenic erectile dysfunction (ED). Bone marrow derived mesenchymal stem cell (BMSC) transplantation was proven effective in treating a neurogenic ED model rat with bilateral CN injury (BCNI). However, stem cell therapy may induce embolization and immunoreactions. We aimed to investigate the effects of filtrated BMSC lysate (BSCL) which does not contain cells.

[Methods] Rats underwent either sham or BCNI surgery. Phosphate buffered saline (PBS) or BSCL was injected postoperatively into the corpus cavernosum (each group; n=7). Erectile function was evaluated by measuring intracavernosal pressure/mean arterial pressure (ICP/MAP) while the CN was stimulated. After the experiment, penis samples were obtained for histological assessment. Neurite outgrowth was evaluated using major pelvic ganglia (MPG), which were placed on Matrigel® and incubated with normal medium or medium with either BSCL or vascular endothelial growth factor (VEGF) as a positive control.

[Results] While BCNI significantly decreased ICP/MAP compared to sham surgery ($P < 0.05$), the injection of BSCL significantly reversed ICP/MAP compared to PBS injection ($P < 0.05$). In addition, MPG treated with medium containing BSCL or VEGF had longer neurite outgrowth than those treated with normal medium. On the other hand, the penile structures of each group did not differ significantly.

[Discussion] Our results suggest that BSCL improve ED caused by CN injury, which may be due to CN regeneration.

1-YIA-26 YIA

FNIII14, an integrin-inactivating peptide, inhibits MUC5AC exocytosis in NCI-H292 airway epithelial cells

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Airway mucus hypersecretion is a hallmark of respiratory diseases. Recently, several reports showed that the extracellular microenvironment regulates mucus hypersecretion; however, its mechanism is not well understood. Therefore, we examined the effect of the integrin inactivating peptide FNIII14 on the production and secretion of MUC5AC, a major component of mucus. In this study, NCI-H292 cells, capable of producing mucus, were treated with TGF-α to induce the production of MUC5AC. FNIII14 did not markedly affect the MUC5AC mRNA expression and the phosphorylation of ERK1/2, a major downstream signaling molecule of EGFR, suggesting that FNIII14 does not affect the production system of MUC5AC. However, the amount of intracellular MUC5AC protein was increased by FNIII14 in a concentration-dependent manner. In addition, intracellular MUC5AC increased by anti-integrinβ1 antibody treatment. These results suggested that secretion of airway mucus is regulated by extracellular matrix and integrins. Furthermore, fluorescence immunostaining revealed that MUC5AC did not co-localize with endoplasmic reticulum or Golgi markers, suggesting that FNIII14 may inhibit the exocytosis system. These findings may lead to the proposal of a new pharmacological mechanism for regulating airway mucus secretion by integrin inactivation.

1-YIA-27 YIA

Basic study to evaluate the risk of liver injury of acetaminophen use in Niemann-Pick disease type C

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Acetaminophen (APAP) is an antipyretic analgesic with high safety, but serious liver damage is observed in overdosed patients or in patients with risk factors. Recently, Niemann-Pick disease type A disease state may exacerbate APAP hepatotoxicity in animal and cellular model due to massive lysosomal lipid storage. Since intracellular cholesterol accumulation also occurs in Niemann-Pick disease type C (NPC), there is a concern that NPC also can be a risk factor for exacerbating the APAP hepatitis. In this study, we examined APAP hepatotoxicity in NPC model hepatocytes (HepG2 cells treated with several concentrations of U18666A (U18), an inhibitor of cholesterol transporter protein NPC) and in NPC model (*Npc1* null) mice. We retrospectively evaluated the changes in liver function when APAP was used in NPC patients.

U18 treatment of cells altered the localization of intracellular cholesterol at all U18 concentrations, but the cholesterol content increased at high concentrations of U18, whereas it decreased at low concentrations of U18. APAP cytotoxicity was increased by both concentrations of U18, APAP cytotoxicity was increased in U18-treated cells in a U18 concentration-dependent manner. Although both concentrations of U18 altered the localization of cellular cholesterol, whereas cholesterol content was decreased in the lower concentrationsuggesting that intracellular cholesterol localization affects APAP toxicity. In mice model, the liver damage after APAP administration was milder in NPC mice than in WT mice. This may be due to the influence of liver inflammation derived from the pathogenesis of NPC on the results.Liver inflammation derived from NPC pathology may affect the results. NPC patient showed no changes in serum indicators suggesting the development of liver damage before and after the APAP use.

Our cellular resultsfindings suggest that abnormal intracellular cholesterol dynamics in NPC can cause exacerbation of APAP liver injury, and this needs to be clarified using other mice model.

1-YIA-28 YIA

Multiplex immunohistochemistry and image cytometry reveals an impact of cochlear resident macrophages on Cisplatin-induced hearing loss

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Background: Cisplatin, or cis-diamminedichloroplatinum (CDDP), is an inorganic member of the platinum-based chemotherapeutic family used to treat different types of malignant tumors. CDDP has been utilized as an important drug for decades, however, it has dose-related serious or irreversible side effects that have a great impact on the patient's quality of life. Previous literature suggested numerous mechanisms for CDDP-induced hearing loss; the proposed mechanisms included oxidative stress to the outer hair cells and DNA damage. However, the exact mechanism of CDDP-induced hearing loss is not clear and its impact on the cochlear immune cells has not been elucidated. Interestingly, recent studies have revealed the existence of macrophages within the cochlea, vestibular system, and the cochlea-vestibular nerve, suggesting that macrophages may be essential for inner ear homeostasis and are a potential target for protection or treatment of hearing loss. In order to understand the role of cochlear macrophages in hearing loss development clearly, we developed multiplex immunohistochemistry (mIHC) and image cytometry for cochlear samples. We investigated the cell population within the cochlea and characterized the various types of cochlear resident macrophages in a CDDP-induced hearing loss mouse model.

Materials and methods: Mice four weeks of age were injected with 5mg/kg/day of CDDP intraperitoneally for six consecutive days. Hearing levels were investigated using auditory brainstem response (ABR) at day 0 prior to CDDP exposure and on days 7 and 14 following CDDP exposure. Mice cochleae were collected at day 0 prior to CDDP exposure and on days 8 and 15 following CDDP exposure and fixed in formalin and paraffin sections before mIHC, which can stain 6 different markers within the same section and identify different macrophage subtypes. Finally, the sections are digitally scanned by nanozoomer digital scanner, and computed image cytometry was applied to these digitally scanned images to precisely interpret the chromogenic signals for the identification and quantification of macrophages.

Results: CDDP exposed mice developed an ABR threshold shift at day 8 post-CDDP which began to recover at day 15 post-CDDP. This threshold shift was associated with a decrease in the number of macrophages of monocyte origin ($F4/80^+$). Additionally, there was an increase in the expression of both pro-and anti-inflammatory macrophage marker ratios in the auditory nerve and spiral ganglia areas on day 8 which also started to resolve on day 15, suggesting a new subcategory of mixed macrophages in the inner ear. Furthermore, the $Iba1^+$ macrophages ratio was increased at day 8 post-CDDP, suggesting microglial activation in the auditory nerve. These findings propose that CDDP exposure causes a state of temporary neuronal inflammation in the auditory nerve and spiral ganglia. This inflammation triggers macrophages polarization towards a new subcategory of macrophages that express markers of M1, M2, and microglia

1-YIA-29 YIA

Nox3-derived superoxide in cochleae induces acquired sensorineural hearing loss

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Sensorineural hearing loss (SNHL) is one of the most common sensory impairments in humans. However, treatment options mostly rely on medical instruments, with no reliable pharmacological interventions. Reactive oxygen species (ROS) produced by NADPH oxidases (Nox) contribute to the development of different types of acquired SNHL, such as drug-induced HL, age-related HL, and noise-induced HL. Although the essential role of Nox3 in otoconia biosynthesis and its possible involvement in hearing have been reported in rodents, immunohistological methods targeted at detecting Nox3 expression in inner ear cells reveal ambiguous results. Therefore, the mechanism underlying Nox3-dependent SNHL remains unclear and warrants further investigation. We generated *Nox3-Cre* knock-in mice, in which Nox3 was replaced with *Cre recombinase* (*Cre*). Using *Nox3-Cre;tdTomato* mice, in which tdTomato is expressed under the control of the *Nox3* promoter, we identified Nox3-expressing regions and cell types in inner ears. Nox3-expressing cells in cochleae included various types of supporting cells (SCs), outer hair cells (OHCs), inner hair cells, and spiral ganglion neurons. Nox3 expression increased with cisplatin, age, and noise insults in specific cell types in cochleae, and resulted in OHC loss (apoptosis). Moreover, increased Nox3 expression in SCs and OHCs, especially at the basal turn of cochleae, played essential roles in acquired SNHL. Thus, we propose that Nox3 inhibition in cochleae is a promising approach to prevent acquired SNHL.

1-YIA-30 YIA

Evaluation of *in vitro* antibacterial activities of therapeutic drugs against *Clostridioides difficile*, and measurement of fecal concentrations in *Clostridioides difficile* infection mouse models.

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[Background, Purpose] Metronidazole (MNZ), vancomycin (VCM), and fidaxomicin (FDX) are standard therapeutic drugs against *Clostridioides difficile* infection (CDI). MNZ resulted in lower clinical cure rate compared with VCM. Detail information about antibacterial activities of the drugs and reasons for MNZ inferiority are not clear. Therefore, we evaluated the *in vitro* antibacterial activities, and measured fecal concentrations in CDI mouse models.

[Method] Minimum inhibitory concentration (MIC) against seven strains of *C. difficile* were determined. Time-kill curves and post-antibiotic effect (PAE) were determined against *C. difficile* ATCC®43255. In addition, fecal concentrations in CDI mouse models were measured.

[Results] MNZ, VCM, and FDX geometric mean MIC were 0.91, 1.81, and 0.34 μg/mL, respectively. MNZ exhibited concentration-dependent and rapid antibacterial activities at low concentrations ranged from 0.5 to 2.0 μg/mL. On the other hands, VCM and FDX exhibited time-dependent and slow antibacterial activities at high concentrations ranged from 0.5 to 32 μg/mL. MNZ showed the shortest PAE (1.9 h). In addition, maximal fecal concentration of MNZ (21.7 μg/g) was significantly lower than that of VCM (222.7 μg/g) at the dose of 40 mg/kg.

[Conclusion] MNZ exhibited noteworthy antibacterial activities against *C. difficile*. However, MNZ PAE was short, and the fecal exposure was significantly small. We think the two characteristics are responsibility for the MNZ inferiority in clinical cure rate.

1-YIA-32 YIA

Effect of advanced glycation end products on STING pathway in macrophage

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2'3'-Cyclic GMP-AMP (cGAMP) is derived from cancer cell and activates innate immune response via stimulator of interferon genes (STING) pathway. STING is an endoplasmic reticulum-localized transmembrane protein and associated with regulation of several signaling pathway. STING bound with cGAMP lead to the phosphorylation of TANK-binding kinase 1 (TBK1). Phosphorylated TBK1 activates interferon regulatory factor 3 (IRF3) and NF-κB, resulting in induction of type 1 interferon and pro-inflammatory cytokines. Type 1 interferon such as IFNβ and CXCL10 has antitumor activity through activation of several immune cells. Therefore, STING pathway play a crucial role in cancer immunity. Advanced glycation end products (AGEs), especially toxic AGEs derived from glycolaldehyde (AGE-3), are biologically reactive compounds associated with diabetic complications and aging-related disorders. Although accumulation of AGEs has been observed in regions of several cancer types, its role for cancer immunity remains unclear. In the present study, we examined the effect of toxic AGEs on the STING pathways in macrophages. In THP-1 cells which is a human monocytic leukemia cell line, cGAMP induced the phosphorylation of TBK1, IRF3 and NF-κB. Glycolaldehyde-derived AGE (AGE3) dose-dependently suppressed cGAMP-induced the phosphorylation of TBK1, IRF3 and NF-κB. These results may suggest that toxic AGE negatively regulate STING pathways of macrophage and suppress cancer immunity.

1-YIA-33 YIA

Mitofusin1 regulates innate immune responses by inhibiting the accumulation of mitochondrial DNA mutation in sepsis

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Mitochondrial dynamics including mitochondrial fusion is implicated in innate immune responses. Here we demonstrate that absence of the mitochondrial fusion protein mitofusin1 (MFN1) enhanced NLRP3 inflammasome-mediated caspase-1 activation and the subsequent secretion of interleukin-1beta (IL-1β) and IL-18 in macrophages. Absence of MFN1 increased the levels of mitochondrial DNA (mtDNA) mutation, but not mtDNA copy number. We further observed that NLRP3 inflammasome activation was enhanced in macrophages from mtDNA mutator mice expressing proofreading-deficient mtDNA polymerase gamma. Both mtDNA mutator mice and myeloid cell-specific *Mfn1* deficient mice displayed increased systemic IL-1β production and mortality in a cecal ligation and puncture (CLP) -induced sepsis. In consistence with our findings from murine models, the levels of mitochondrial heteroplasmy, the co-existence of wild type and mutated mtDNA, in human white blood cells were significantly associated with disease severity of critically ill patients and increased in patients with sepsis. Collectively, our study demonstrates that MFN1 regulates NLRP3 inflammasome-dependent inflammation in sepsis by preventing the accumulation of mtDNA mutation.

1-YIA-34 YIA

Analysis of the brain-periphery immune system by the specific manipulation of stress-related neurons

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Our body maintains homeostasis when we are exposed to external and internal stimuli. However, chronic stress changes the sympathetic and parasympathetic nervous systems as well as endocrine systems such as the hypothalamic-pituitary-adrenal (HPA) axis, which leads to a loss of homeostasis. This maladaptation triggers several kinds of disorders, like major depressive disorder (MDD), psychosomatic disease, and immune dysfunction. In addition, excess stress is known to affect the function of several brain areas, including the hypothalamic paraventricular nucleus (PVN), which plays a critical role in the adaptation to stress. In particular, corticotropin-releasing hormone (CRH) neurons are most strongly implicated in the stress response because the HPA axis is activated by facilitating CRH-containing neurons. Although the activation of CRH^{PVN} neurons may play a role in immune cell homeostasis, little is known about how the direct activation of CRH^{PVN} neurons may suppress immune cells. In this study, we investigated whether the specific activation of CRH^{PVN} neurons by DREADD systems could affect the peripheral immune system. As a result, the number of $CD4^+$ T cells and NK cells was influenced by the activation of CRH^{PVN} neurons. Furthermore, there were correlations between the immune cell population and plasma cortisol levels. Taken together, these findings provide further evidence that the modulation of stress-associated PVN-CRH neurons may affect the peripheral immune system via the HPA axis.

1-YIA-35 YIA

Anti-asthmatic effect of nanoliposomal ceramides in a mouse model

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【Objective】Ceramide has been emerging as an anti-inflammatory lipid, and nanoscale delivery system of ceramides is a potential therapeutic strategy for inflammatory diseases. In this study, we evaluated the therapeutic effect of ceramide formulation (nanoliposomal ceramides) on allergic asthma in a mouse model.

【Methods】BALB/c mice sensitized with ovalbumin (OVA) were treated with nanoliposomal ceramides or ceramide-free liposomes followed by multiple OVA challenges. The numbers of mononuclear cells, eosinophils and neutrophils infiltrated in bronchoalveolar lavage fluids were counted. Airway remodeling was evaluated by staining with PAS and Masson trichrome. Type 2 cytokines in homogenized right lungs were measured by ELISA.

【Results】Treatment with nanoliposomal ceramides suppressed OVA-induced infiltration of mononuclear cells, eosinophils and neutrophils into the lung and development of airway remodeling. However, OVA-induced production of type 2 cytokines was not changed by nanoliposomal ceramides.

【Conclusions】Nanoliposomal ceramides are suggested to have anti-asthmatic effects by suppressing the development of airway remodeling. Our study gives insight into the development of ceramide-based therapy for allergic asthma.

1-YIA-36 YIA

The automated scratching detection method of mice using artificial intelligence

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Since the scratching assessment is the only way to estimate itching sensation in non-verbal experimental animal, it is utilized in the various research fields. However, current methods depend on human observation, which is laborious, low-throughput, and includes observer-bias. We here aimed to establish an automated scratching detection method of mice using neural network, an artificial intelligence technology which excels in image recognition.

Scratching was elicited by intradermal injection of a pruritogen, lysophosphatidic acid to BALB/c mice and their behavior was recorded with a video camera. Frame images were obtained from video data and classified into two classes: scratching or not. We then trained convolutional recurrent neural network (CRNN) with labeled datasets. Trained CRNN predicted scratching of first-look data with high accuracy (sensitivity: 81.6%, positive predictive rate 87.9%). We confirmed that the number and duration of predicted scratching bouts were comparable to those of human observation. Trained CRNN could also successfully detect scratching evoked by hapten-induced atopic dermatitis (sensitivity: 94.8%, positive predictive rate: 82.1%).

We here established a novel automated scratching detection method using artificial intelligence, which is applicable to the assessment of pathological mouse model.

1-YIA-37 YIA

P2X4 receptor signaling potentiate Mas-relate G protein-coupled receptor B2 induced mast cell activation.

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Mas-relate G protein-coupled receptor b2 (Mrgprb2) and its human ortholog MRGPRX2 are specifically expressed in mast cells and widely recognizes cationic ligands such as neuropeptides, bacterial components and some drugs including vancomycin and morphine. These ligands of Mrgprb2/MRGPRX2 involved in (pseudo) allergic reactions, pain and infectious diseases. Previously, we reported that extracellular ATP potentiates antigen-induced mast cell degranulation *via* P2X4 receptor activation. In this study, we investigated the effect of P2X4 receptor signaling on degranulation induced by Mrgprb2 activation. Stimulation of mouse peritoneal mast cell (PMC) with Mrgprb2 agonist compound 48/80 (C48/80) induced degranulation in a concentration-dependent manner. C48/80-induced degranulation was potentiated by ATP but not by ADP, UTP and UDP. Similar results were obtained with another Mrgprb2 agonists substance P and PAMP-12. The potentiation by ATP was absent in PMC prepared from P2X4 receptor deficient mice. These results suggest that Mrgprb2-mediated mast cell activation is potentiated by the P2X4 receptor signaling.

1-YIA-38 YIA

The role of PGD_2/CRTH2 signaling in allergic reaction

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【Background & Aim】 Antigen specific IgE is a fundamental factor in allergic reaction. In allergic reaction, prostaglandin D_2 (PGD_2) is known to play crucial role. PGD_2 acts on (chemoattractant receptor-homologous molecule on Th2 cells) CRTH2 receptor and its signaling is known to exacerbate allergic reaction by promoting antigen specific IgE production. However, it still be unknown the roles of PGD_2/CTH2 signaling in the production of antigen specific IgE. We aimed to reveal the role of PGD_2/CRTH2 signaling in allergic reaction.

【Methods & Results】 We sensitized wild type (WT) and CRTH2 deficient mice (*Crth2*$^{-/-}$) with ovalbumin (OVA) intradermally. The titer of OVA specific IgE in serum was lower in *Crth2*$^{-/-}$ than WT after the sensitization. In the draining lymph node (dLN), the percentage of T follicular helper (Tfh) cells, a critical regulator of IgE production, was lower in *Crth2*$^{-/-}$ than WT. These results suggested that CRTH2 signaling promotes differentiation of Tfh cells and IgE production in dLN. Intradermal administration of OVA increased the concentration of PGD_2 in the LN of WT. Immunostaining showed that the synthase of PGD_2, hematopoietic prostaglandin D synthase (HPGDS), was expressed in dendritic cells (DCs) in LN. Bone marrow derived dendritic cells released PGD_2 in response to OVA stimulation. These results suggested that antigen stimulation increased PGD_2 production in the DCs in dLN.

【Conclusion】 We found that PGD_2 derived from DC promotes antigen specific IgE production through CRTH2 signaling mediated Tfh differentiation in LN.

Spontaneous and local ATP release in astrocytes revealed by spatiotemporal analysis using two-photon microscopy

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Astrocytes, a non-neuronal cell in the central nervous system, participate in the purinergic signaling. As lowered ATP release from astrocytes is related to depressive-like behavior, basal ATP transmission is thought to play important roles in physiological brain function. Since a single astrocyte interacts with up to 140,000 synapses in rodents, spatial property of ATP release is essential to understand purinergic signaling within brain architecture, which is not well understood. Using two-photon microscopy, we investigated spatiotemporal properties of ATP release in astrocytes with a novel genetically encoded extracellular ATP sensor, $GRAB_{ATP}$. In neuron-astrocyte coculture, TTX insensitive ATP release events were detected. In acute slices in which astrocytes expressed $GRAB_{ATP}$ sparsely by means of *in utero* electroporation, we also observed spontaneous ATP release events, which were suppressed by astrocyte specific toxin, fluorocitrate, and insensitive to TTX and vesicular release blocker, bafilomycin A1. Typical ATP release spread over 50–200 μm^2 with concentration roughly ranged 0.5–5 μM. Simultaneous monitoring with intracellular calcium revealed that ATP release and Ca^{2+} event rarely co-occurred. In conclusion, our findings indicate that astrocytes spontaneously release ATP in acute slices via mainly non-vesicular Ca^{2+} independent pathway, which possiblly activates purinergic receptors in nearby hundreds of synapses.

Regulation of neuron-astrocyte communication by microglia

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Astrocytes become reactive upon injury and inflammation in the brain to alter their molecular profiles, morphologies and functions. Reactive astrocytes alter the expression of receptors which are responsible for their functions especially communications in neuron-glia and glia-glia. Among such receptors, the expression of P2Y1 receptors (P2Y1) is upregulated in many neurological diseases including epilepsy and Alzheimer's disease, in which neuronal hyperexcitability is commonly observed. We have previously shown that P2Y1 upregulation in astrocytes trigger neuronal hyperexcitability by enhancing neuron-astrocyte communications. However, the mechanism underlying the upregulation of P2Y1 in astrocytes remains unknown. We investigated the role of microglia in enhanced P2Y1R signaling in astrocytes during pathological conditions. To ask whether microglia play a role in P2Y1 upregulation in astrocytes, we depleted microglia by treatment with PLX5622 and found much larger Ca^{2+} elevation evoked by a P2Y1 agonist and more *P2ry1* transcripts in astrocytes. Microglia depletion enhanced extracellular ATP level presumably through impairment of degradation of ATP. These findings suggest that microglia should has an important role to control P2Y1 receptor expression in astrocytes and negatively regulate neuron-astrocyte communication.

Microglia repopulation ameliorates the pathology of Alexander disease, the primary astrocyte disease.

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Alexander disease (AxD) is a rare neurological disorder caused by mutations of *GFAP* gene, an astrocyte selective intermediate filament, and AxD astrocytes show abnormal aggregates, i.e., Rosenthal fibers (RFs), a main pathological finding. AxD brain also shows neuroinflammation, where microglia are activated. In this study, we show that manipulation of microglia would be a potential therapeutic strategy for the treatment of AxD using AxD model mice with human GFAP mutation (R239H) (60TM), To achieve this, we used an ON/OFF protocol of PLX5622 (PLX), a selective CSF-1 receptor antagonist. PLX-ON depleted almost all resident microglia, and subsequent PLX-OFF caused almost complete recovery of microglia, indicating that the old microglia should be replaced with newly repopulated microglia in the AxD brain. The amount of RF stained with Fluoro Jade B was significantly reduced by this replacement, suggesting that microglia should become more protective by their repopulation. Furthermore, microglial replacement significantly decreased the expression of Lipocalin2, the most upregulated molecule in 60TM astrocyte, and other inflammatory molecules in 60TM. Taken together, the ON/OFF protocol of PLX allows us to replace old microglia with newly repopulated ones in the AxD brain, which can dramatically ameliorate AxD pathogenesis.

Contribution of oligodendrocyte precursor cells to disease severity in a mouse model of multiple sclerosis

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Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease. During demyelination, oligodendrocyte precursor cells (OPCs) can proliferate, migrate to the site of injury, differentiate into mature oligodendrocytes, and generate new myelin. Since increased numbers of OPCs are observed in MS lesions, insufficient differentiation of OPCs is considered to be one of the causes of demyelination and axonal degeneration. However, it has been reported that OPCs could promote the disruption of the BBB or release inflammatory cytokines under inflammatory conditions, and the involvement of OPCs in MS is not fully understood. In this study, we investigated the role of OPCs in the acute phase of MS by removing OPCs. We employed a mouse model of MS, experimental autoimmune encephalomyelitis (EAE), which was induced by immunization with myelin oligodendrocyte glycoprotein (35-55). We depleted OPCs by intraperitoneally injecting tamoxifen and diphtheria toxin (DT) in *Pdgfra*$^{CreER/+}$:*Rosa26*$^{DTR/+}$ mice. When DT was injected from the next day of disease onset, EAE severity was significantly reduced in OPC-depleted mice. Quantitative RT-PCR analysis revealed that the expression levels of pro-inflammatory cytokines and the marker of helper T cell subset Th17 were suppressed in the spinal cord of OPC-depleted group. These data suggest that OPCs are involved in CNS inflammation, T cell response and the development of EAE.

Lack of $P2Y_1$ receptors in Müller cells causes a glaucoma-like phenotype

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Glaucoma is a leading cause of blindness worldwide, which is caused by the degeneration of retinal ganglion cells (RGCs). An elevated intraocular pressure (IOP) is widely recognized as one of the major risk factors for glaucoma. We have reported that purinergic $P2Y_1$ receptor is essential for IOP reduction and $P2Y_1$ receptor knockout ($P2Y_1$KO) mice show hypertensive glaucoma-like phenotype. We already reported that $P2Y_1$ receptor is located in ciliary body(CB) and trabecular meshwork (TM), and controls production and outflow of aqueous humor, respectively. We also found that ocular hypertension itself did not solely cause RGC degeneration at young ages, so we hypothesized IOP-independent pathogenic mechanism. Immunohistochemical analysis revealed that $P2Y_1$ receptors were expressed in Müller cells in the retina in addition to CB and TM. To test the role of $P2Y_1$ receptors in Müller cells for RGC damages, we made *Mlc1*-tTS::*P2ry1*$^{tetO/tetO}$ mice (Müller cell-specific $P2Y_1$ receptor conditional knockout mice; MC-cKO). We measured the IOP of control (*P2ry1*$^{tetO/tetO}$) and MC-cKO mice and found no difference between them. Next, RGC damage was compared by counting the number of Rbpms-positive cells. Howeve, MC-cKO mice at 12 months old showed significantly higher number of RGCs loss than that in age-matched control mice. Accompanied this, the number of apoptotic cells significantly increased in the MC-cKO mice. Taken together, our results demonstrated that the lack of $P2Y_1$ receptors in Müller cells promotes RGC loss without IOP elevation, suggesting an importance of Müller cells for pathogenesis of glaucoma. (1303/1350 words)

Developmental synchronous firing modulates synaptic connectivity

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During the critical period of development of the central nervous system, synaptic connections are first excessively generated and then reduced gradually via selective synaptic pruning. However, the rule how select the target synapses of pruning remains incompletely known. The candidate mechanisms include the Hebb's rule, which suggests that synaptic connections between neurons that fire synchronously would survive from developmental pruning. To experimentally verify this well-known theory, we developed a new method that enables to induce synchronous firing in in vivo layer 2/3 neurons of the mouse somatosensory cortex that sparsely expressed channelrhodopsin2 (ChR2) through in utero electroporation. We transcranially stimulated ChR2-positive neurons on postnatal days 9-to-13 and measured the connection probability between these neurons using in vitro patch-clamp recordings on postnatal days 21-to-28. The neocortex that received chronic photostimulation exhibited higher probabilities of synaptic connections between ChR2-positive neurons, compared to non-stimulated neocortex. The results are consistent with the Hebb's rule.

Localization of Desmoplakin, an adhesion-related molecule, in the dentate gyrus of mouse hippocampus

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The desmosome is an intercellular junctional structure that provides strong adhesive forces between cells. Desmoplakin, an integral core protein of the desmosome, is expressed not only in epithelium and cardiac muscle but also in hippocampal neurons, which do not have desmosomes. The desmoplakin and the plakoglobin in cultured hippocampal neurons is shown to be coimmunoprecipitated with N-cadherin. However, there is still little evidence for the localization or function of desmoplakin in hippocampal neurons. Here, we found that three variants of *Desmoplakin* are expressed in the hippocampus. One of them corresponded to *Desmoplakin Ia*, which has only been identified in humans. We also confirmed that *Desmoplakin* mRNA is expressed in mature granule cells in the dentate gyrus of the mouse hippocampus by *in situ* hybridization. Desmoplakin immunoreactivity was localized to ciliary structures and dendrites of the granule cells. Exploration of the specific function of Desmoplakin in the granule cells of dentate gyrus may provide insight into the architecture and plasticity of hippocampal neural network.

Dynamic changes in orexin activities associated with reward-based motivative behavior

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Orexin neurons in the hypothalamus regulate physiological functions, including energy homeostasis and wakefulness, and are also related to motivation. Here, we examined the roles of orexin neurons in motivated behaviors. We measured the activities of orexin neurons using fiber photometry under a free-moving condition, in which the rats were subjected to the fixed ratio (FR) or progressive ratio (PR) schedule of a touchscreen-based automated operant task. To measure the activities of orexin neurons, AAV-FLEX-GCaMP7s was injected into the hypothalamus of Orexin-Cre rats. We found that under FR5 conditions in which rats were able to obtain a food pellet by touching the screen consecutively five times, the activity in orexin neurons was increased after the fifth screen touch (after which one food pellet is delivered). The activity peaked before rats obtained reward, and then decreased after food intake. Next, we included non-reward trials in the FR5 test in which the rat was not able to earn reward even after touching the screen five times. The orexin activities in non-reward trials were also increased after the fifth screen touch, but the decrease after food intake was diminished compared to those in reward-trials. In the PR schedule test, the orexin activities were gradually increased. Together, these observations suggest that the orexin activities are associated with motivational behaviors, and that orexin neurons may be involved in craving and reward prediction, and satisfaction.

The effect of neurotrophin-3 overexpression on adult hippocampal neurogenesis

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It is known that stress suppresses neurogenesis in the hippocampus. Neurotrophin-3 (NT-3), a neurotrophic factor, has been reported to be upregulated in the hippocampus of adult mice by stress and corticosterone administration. In order to investigate the effects of increased NT-3 on neurogenesis in the hippocampus and stress-induced behaviors, we generated NT-3 overexpressing mice in the hippocampus by administering adeno-associated virus carrying the NT-3 gene (AAV-NT-3). NT-3 mRNA was expressed more than 7 times higher in the hippocampus by AAV-NT-3 administration compared to control hippocampus. NT-3 expression was mainly localized in hippocampal hilus. After 4 weeks of AAV-NT-3administration, the number of proliferating cells in the hippocampal dentate gyrus was decreased in the NT-3 overexpression group compared to the control group. This result suggests that high dose of NT-3 may suppress proliferation of neuronal stem cells/progenitors in the dentate gyrus. In the future, we plan to investigate changes in neural differentiation and maturation in the hippocampus of NT-3 overexpressing mice to further clarify the role of NT-3 in neurogenesis. It is also necessary to clarify the effects of NT-3 on stress by examining stress-induced anxiety-like and depression-like behaviors in NT-3 overexpressing mice.

An *in vitro* model of thalamocortical and corticothalamic interactions using human induced pluripotent stem cells

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Interactions between the thalamus and cerebral cortex are crucial for relaying sensory signals, and their impediment is associated with neuropsychiatric disorders. However, the pathogenesis of these disorders, including autism spectrum disorder, remains unsolved due to the lack of *in vitro* models that mimic pathophysiological events of the human brain. Brain organoids, three-dimensional cell aggregates differentiated from pluripotent stem cells, have been shown to partly mimic the structure, function, and development of some brain regions *in vitro*. Here we report *in vitro* thalamocortical and corticothalamic interactions by generating assembloids, a 3D assembly of organoids, from human induced pluripotent stem cells (hiPSCs). We differentiated hiPSCs to both thalamic organoids and cortical organoids, each of which expressed brain region-specific markers. We then generated assembloids by fusing the thalamic and cortical organoids. Labeling the organoids with fluorescent proteins visualized reciprocal projections in the assembloids. In addition, rabies viral tracing demonstrated transsynaptic labeling between two organoids, suggesting the formation of synaptic connections in the assembloids. The *in vitro* models of neural circuits between the thalamus and cortex will help us understand neuropsychiatric disorders.

Regulatory mechanism of stress sensitivity by Shati/Nat8l in the dorsal striatum

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[Background] Chronic stress does not trigger depression in all individuals, as some remain resilient. However, the underlying mechanisms that contribute to stress sensitivity have been poorly understood. We found that Shati/Nat8l, N-acetyltransferase, levels increased in the dorsal striatum of stress-susceptible mice exposed repeated social defeat stress (RSDS). In the present study, we revealed the mechanism of regulation in stress sensitivity by Shati/Nat8l.
[Methods] C57BL/6J male mice were exposed RSDS using ICR mice, and the susceptible or resilient group were classified by social interaction test. We generated dorsal striatal Shati/Nat8l overexpression (STR-Shati OE) or knockdown mice (STR-Shati KD). These mice were assessed depression-like behaviors after RSDS. We investigated the relationship between Shati/Nat8l and serotonin in the striatum by pharmacological regulation of serotonergic system and in vivo microdialysis.
[Results] Striatal serotonin decreased in stress susceptible, not resilient mice. STR-Shati OE showed the vulnerability to social stress. Conversely, STR-Shati KD showed the resilience to social stress. The reduction of striatal serotonin was observed in STR-Shati OE. The vulnerability to stress in Shati OE recovered by modulation of serotonergic system.
[Conclusions] Striatal Shati/Nat8l controls stress sensitivity via regulation of serotonin in the striatum. Our study suggested the novel mechanisms underlying stress sensitivity, and striatal Shati/Nat8l could be a new target for medical tools for depression.

The L-DOPA receptor GPR143 in the indirect pathways regulates an anxiety-like behavior in mice

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We propose that L-DOPA by itself is a neurotransmitter. Recently, a G-protein coupled receptor GPR143, a gene product of ocular-albinism1, was identified as a receptor for L-DOPA. In this study, to identify the physiological role of GPR143, we examined the phenotypic analysis using Gpr143-gene deficient (GPR143-KO) mice. We found that time spent in open arms using zero-maze test was decreased in GPR143-KO mice when compared to wild-type (WT) mice. The time spent in open arms was also decreased in indirect pathway striatal neuron specific GPR143-KO mice. To investigate the involvement of endogenous L-DOPA in this phenotype, we perform zero-maze test after treatment with alpha-methyl-para-tyrosine (α-MPT), a synthetic inhibitor of L-DOPA. Intraperitoneal injection of α-MPT at the dose of 3 mg/kg, which decreased the striatal content of L-DOPA without affecting that of dopamine, suppressed the time spent in open arms in WT mice. This effect of α-MPT was not observed in GPR143-KO mice. These results suggest that L-DOPA regulates anxiety-like behavior through GPR143 expressed in the striatal indirect pathway neurons.

1-SS-13 Student Sessions

Roles of synaptic mitochondrial regulations for stress susceptibility

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Chronic social stress induces neuronal dysfunctions in the medial prefrontal cortex (mPFC) for emotional and cognitive disturbances. However, the subcellular mechanism remains elusive. Here we examined ultrastructural and multi-omics changes in the mPFC in a mouse model of social defeat stress. Acute stress induced dendritic membrane deformation with mitochondrial swelling in mPFC neurons, leading to dendritic atrophy after chronic stress. Synaptic, but not bulk tissue, proteomes in the mPFC differentiated naïve and stressed mice and further uncovered two distinct states in stressed mice. Proteins involved in mitochondrial metabolic functions mostly decreased with chronic stress regardless of the synaptic proteomic state. By contrast, proteins responsible for mitochondrial homeostasis increased in stressed mice with a specific synaptic proteomic state associated with behavioral resilience to chronic stress. These findings suggest that the balance between mitochondrial metabolic dysfunction and its maintenance at mPFC synapses determines stress susceptibility in mice.

Possible involvement of hypothalamic paraventricular nucleus PACAP in chronic pain-induced negative emotional responses in mice

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Recent evidence has suggested that pituitary adenylate cyclase-activating polypeptide (PACAP) has critical roles in central and peripheral pathways, such as spino-parabrachio-amygdaloid and hypothalamic-pituitary-adrenal pathways, mediating stress-related negative emotional behaviors. Although it is well established that there is a great degree of comorbidity of chronic pain and negative emotional behaviors, the cellular mechanism underlying chronic pain and anxiety/depression interaction still remains to be elucidated. Here, we evaluated possible involvement of PACAP signaling in the development of anxiety- and depression-like behaviors after peripheral nerve injury in mice. We observed that spinal nerve ligation (SNL) induced anxiety- and depression-like behaviors lasting for at least 3 weeks in wild-type (PACAP +/+) mice. However, the development of SNL-induced anxiety- and depression-like behaviors was almost completely abrogated in PACAP -/- mice. Furthermore, we found that selective overexpression of PACAP by the infection of adeno-associated virus in the hypothalamic paraventricular nucleus (PVN), but not neighboring ventromedial hypothalamus, region resulted in the induction of anxiety-like behavior. In contrast, siRNA-mediated knockdown of PVN PACAP attenuated the development of SNL-induced anxiety- but not depressive-like behavior. Our data support that PVN PACAP signaling is involved in an important mechanism underlying the anxiety-like behaviors in peripheral neuropathic pain condition.

A Novel PACAP receptor PAC1 antagonist exhibits fast and lasting anti-depressant effect

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Psychiatric disorders, such as depression and anxiety related disorders, posed a significant burden worldwide. Therefore, it is necessary to develop additional safe and effective antidepressants. Accumulating evidence indicates that PACAP (pituitary adenylate cyclase-activating polypeptide) and its preferring receptor PAC1 are involved in psychiatric disorders, especially stress-related disorders. Recently, we developed novel small-molecule, non-peptide, and high-affinity PAC1 antagonists and showed that the antagonists significantly attenuated mechanical allodynia in mice. In this study, we aimed to characterize the PAC1 antagonist as a new therapeutic reagent for stress-related disorders and conducted behavioral pharmacological experiments in mice. A single dose of the PAC1 antagonist significantly improved anxiety-like and depressive-like behaviors in chronic social defeated stress mice, and this effect lasted long period which was similar to that of ketamine. In addition, the PAC1 antagonist did not exhibit behavioral impairments, including pre-pulse inhibition deficits and cognitive deficits in naïve control mice. These results indicate that the novel PAC1 antagonist may have a robust antidepressant effect and highly safe profile.

1-SS-16 Student Sessions

Deep learning of mother-infant interactions in V1b Vasopressin receptor knockout mice

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【Background】Oxytocin, a neurohypophysial hormone from the posterior pituitary, is known as an important factor for childcare and breastfeeding. Also, vasopressin has been reportedly involved in maternal behaviors through the vasopressin receptors of V1A and V1B subtypes. Previous studies demonstrated that the V1A receptor antagonist given into the median preoptic area of rat resulted in significant reduction of caregiving behavior of the mother. Although the studies on the oxytocin hormone and vasopressin/V1A receptor have been extensively conducted, our knowledge on the V1B receptor in maternal behavior is still limited.【Purpose】We intended to clarify a role of the V1B receptor in mother-child interaction during lactating period.【Methods】We compared exploratory behavior between nonpregnant females of control mice and those of the V1B knockout mice in open field test. After giving a birth, the mothers were examined on their behavior in pup retrieval test. Moreover, massive amounts of data from behavioral recordings were visualized and mother-infant relationship was analyzed by deep learning strategy.【Results and discussion】After training about 3000 images, our deep learning model successfully classified mother and babies with 99% accuracy. The analysis results by deep learning model were in good agreement with the observational results by an investigator. Together, we propose that this new method can be applied further to other areas of behavioral study to overcome the limitations and increase the efficiency for analyzing complex behaviors.

Treatment with lysophosphatidic acid（LPA）improves depressive behaviors in neuropsychiatric lupus erythematosus (NPSLE) model mice through LPA receptor

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【Objective】NPSLE is an intractable autoimmune disease with neuropsychiatric symptoms, such as depression. Recent, studies reported that LPA reduces neuroinflammation. MRL/lpr mouse has been used as an animal model of NPSLE because of behavioral abnormalities. In this study, we examined the effects of LPA on NPSLE model mice.

【Methods】15-week-old MRL/lpr mice were treated with or without LPA for 2 weeks. In another study, the mice were pretreated with or without ki16425 (an antagonist of LPA receptors 1 and 3) and they were treated with LPA for 2 weeks. After treatment, the behavioral tests were performed as indices of depression. Histological examinations were performed in the harvested brain tissues.

【Conclusions】The treatment with LPA significantly reduced the depressive behaviors in MRL/lpr mice. Pretreatment with ki16425 negated the effects of LPA. The expressions of Iba1 and CD68 (microglial markers) were increased in the hippocampus and prefrontal cortex of MRL/lpr mice compared to controls and LPA treatment suppressed the increases of Iba1 and CD68. Pretreatment with ki16425 negated the inhibitory effects of LPA. These findings suggest that LPA receptor stimulation by LPA may suppress microglial activation and depressive behaviors in NPSLE model mice.

Activation of δ-opioid receptors in the infralimbic cortex and amygdala facilitates contextual fear extinction in mice.

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Facilitation of fear extinction is expected to shorten the duration of treatment for fear-related disorders. Previously, we found that selective agonist of δ-opioid receptor (DOP), KNT-127, facilitates extinction learning of contextual fear. Here, we investigated the brain regions which mediate the action of KNT-127 on fear extinction in mice. On day 1, male C57BL/6J mice were contextually conditioned with 8 foot-shocks. On day 2, the mice were re-exposed to the conditioning chamber for 6 min as an extinction training (re-exposure 1). KNT-127 was microinjected into the amygdala (AMY), hippocampus (HPC), prelimbic (PL) and infralimbic (IL) sub-regions of the medial prefrontal cortex, 30 min before re-exposure 1. On day 3, mice were re-exposed to the chamber for 6 min as a memory testing (re-exposure 2). As a result, KNT-127 (50 ng/mouse) into the AMY and IL, but not HPC and PL, significantly reduced freezing behavior in re-exposures 1 and 2. These effects of KNT-127 in the AMY and IL were abolished by pretreatment with a selective DOP antagonist naltrindole (NTI). Further, MEK/ERK inhibitor, U-0126, blocked the effect of KNT-127 in the AMY. These results suggested that KNT-127 facilitated extinction learning via DOPs in the AMY and IL, and that MEK/ERK pathway in the AMY mediates the extinction-facilitating action of KNT-127.

Involvement of GPR143, an L-DOPA receptor, in haloperidol-induced extrapyramidal symptoms

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We propose that L-DOPA by itself is a neurotransmitter. Recently, a G-protein-related receptor (GPCR) GPR143, a gene product of ocular-albinism1, was identified as an L-DOPA receptor. We previously showed that non-effective dose of L-DOPA potentiates behavioral response to quinpirole, a dopamine D2 receptor (D2R). However, it remains undetermined whether and how GPR143 regulates D2R-mediated behaviors. In this study, we analyzed behavioral responses to several D2R ligands using *Gpr143* gene-deficient (GPR143-KO) mice. We found that haloperidol, a D2R antagonist (0.5mg/kg)-induced catalepsy was attenuated in GPR143-KO mice when compared to wild type (WT) mice. To clarify which neuron circuits are responsible for this phenotype, we investigated haloperidol-induced catalepsy using mice that expressing cre recombinase in D2R-, adenosine A2a receptor (indirect pathway)-, choline acetyltransferase (cholinergic interneuron)-positive neurons. Haloperidol-induced catalepsy was attenuated in D2R-cre (+); $Gpr143^{flox/y}$ and ChAT-cre; $Gpr143^{flox/y}$ mice. These results suggest that GPR143 expressed in the striatal cholinergic interneurons plays an important role in haloperidol-induced catalepsy.

Effect of hachimijiogan on social cognitive dysfunction and arginase-1 expression in SAMP8 mice

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Hachimijiogan (HJG) is traditional herbal medicine. Recently, it has been reported that cognitive dysfunction in patients with Alzheimer's disease (AD) was improved by HJG treatment. However, the mechanism by which HJG improves cognitive dysfunction is still unclear. Senescence-accelerated mouse prone 8 (SAMP8) mice are used as a spontaneous animal model of AD. The present study examined the effect of HJG on cognitive dysfunction and glial cell marker expression in SAMP8 mice.

SAMP8 mice were orally administered HJG (1000 mg/kg/d) from 12 weeks of age. Three-chamber sociability and social novelty test were conducted at 38-39 weeks of age. The glial cell marker levels in the hippocampus were analyzed by western blotting.

Vehicle-treated SAMP8 mice showed the impairment of social cognition compared with SAMR1 mice, which are resistant to senescence. On the other hand, HJG-treated SAMP8 mice did not show significant impairment compared with SAMR1 mice. The levels of arginase-1, a protective microglia marker, were significantly decreased in vehicle-treated but not HJG-treated SAMP8 mice.

Our findings suggest that social cognitive dysfunction of SAMP8 mice is due, in part, to the reduced protective microglia. HJG may make the progression of cognitive dysfunction slower by attenuating reduction in protective microglia.

Mirogabalin inhibits paclitaxel-induced mechanical allodynia in mice by acting on $\alpha_2\delta$-1 subunit in the spinal dorsal horn

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Chemotherapy-induced peripheral neuropathy (CIPN) is a complication caused by several anti-cancer drugs, which profoundly affects the patient's quality of life. Paclitaxel (PTX) is used in the treatment of common cancers, and usually causes dysesthesias, paresthesias and numbness, hypersensitivity to mechanical stimuli, that patients frequently suffer in feet and hands. Mirogabalin (MGB) has been developed as a novel gabapentinoid, and its analgesic effect is exerted by binding to the $\alpha_2\delta$-1 subunit of voltage-gated calcium channels. Although MGB is used for the treatment of peripheral neuropathic pain including diabetic peripheral neuropathy and postherpetic neuralgia, no clinical studies have been reported in CIPN. Here, we conducted an investigate the effects of MGB on PTX-induced peripheral neuropathic pain. A single oral administration of MGB dose-dependently inhibited PTX-induced mechanical allodynia but did not affect locomotor activity. Next, we administered MGB topically and found that intrathecal injection suppressed mechanical allodynia, but intradermal injection into footpad did not. In fact, $\alpha_2\delta$-1 protein expression was increased in the spinal cord on the PTX model. Together, these results suggest MGB inhibits PTX-induced mechanical allodynia by acting on $\alpha_2\delta$-1 subunit in the spinal dorsal horn.

Pregabalin as a pathogenic mechanism-based therapeutic strategy for dry eye-induced chronic ocular pain

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Dry eye-induced chronic pain involves hypersensitivity and hyperalgesia, and is a clinically serious problem. However, effective therapeutic approach has not been established other than eye drops to alleviate the symptoms. Aiming at developing a pathogenic mechanism-based therapeutic strategy, we performed experiments using a rat dry eye model with lacrimal gland excision (LGE). On the LGE side, corneal hypersensitivity and hyperalgesia were developed. In the trigeminal nucleus of the LGE side, neuronal hyper-activation, transient activation of microglia, persistent activation of astrocytes, upregulation of the voltage-dependent Ca^{2+} channel $\alpha_2\delta$-1 subunit were observed. Next, we evaluated the efficacy of ophthalmic treatment for corneal damage and pregabalin, a ligand for $\alpha_2\delta$-1 subunit, after chronic pain was established in LGE rats. Ophthalmic treatment alone was not effective for hyperalgesia. In contrast, the combination of ophthalmic treatment and pregabalin effectively abrogated hyperalgesia, neural activity, the upregulated $\alpha_2\delta$-1 subunit, and activated astrocytes. These results highlight a crucial role of $\alpha_2\delta$-1 subunit upregulation in the trigeminal nucleus as a pathogenic mechanism and the therapeutic target for dry eye–induced chronic pain.

Inhibitory effect of mirogabalin for chronic itch in atopic dermatitis model mice

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Chronic itch is an unpleasant sensation and reduces quality of life. Especially, atopic dermatitis (AD) is representative disease with chronic itch. The itching associated with a nettle rash is potently alleviated by H_1 receptor antagonists, but that with AD is not. *Nevertheless,* its underlying mechanisms are poorly understood. Here, using AD model mice, we showed inhibitory effect of the novel gabapentinoid, mirogabalin on spontaneous scratching behavior. The number of scratch bouts were increased in AD model mice as compared with healthy mice. These spontaneous scratching were not suppressed by H_1 receptor antagonist. Next, we examined the effect of mirogabalin (10 mg/kg) by oral administration in AD model mice, and mirogabalin suppressed scratch bouts. We also examined sedation by using healthy mice with Rota-Rod test and showed mirogabalin (10 mg/kg) did not have sedation. Furthermore, we examined the effect of other gabapentinoids. Gabapentin (100 mg/kg) and pregabalin (30 mg/kg) also inhibited scratch bouts in AD model mice. These results suggest that mirogabalin may be effective against chronic pruritus in AD.

Role of astrocytes in dysfunction of spinal dorsal horn neurons crucial for neuropathic allodynia

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Mechanical allodynia is one of the symptoms of neuropathic pain and is produced by tactile stimulation. Recently, we have identified a subset of spinal dorsa horn (SDH) inhibitory interneurons that is operated by adeno-associated viral (AAV) vectors incorporating a neuropeptide Y promoter (AAV-NpyP^{+}). In a model of neuropathic pain caused by peripheral nerve injury (PNI), these neurons exhibit deeper resting membrane potentials, and their excitability is impaired, which are necessary for neuropathic allodynia. However, the mechanism underlying these changes remains unknown. In this study, we show that the dysfunction of AAV-NpyP^{+} neurons require astrocytes that are activated in the SDH after PNI. We found that inhibition of PNI-induced activation of SDH astrocytes by expressing a dominant negative form of STAT3 (dnSTAT3: an inactive mutant STAT3) alleviated Aβ fiber-derived neuropathic allodynia and normalized alterations in resting membrane potentials and excitability of AAV-NpyP^{+} neurons. Our findings suggest that suppressing activation of astrocytes after PNI restores normal AAV-NpyP neurons activity and attenuates neuropathic allodynia. Thus, inhibiting astrocytes activation may be a new therapeutic target for neuropathic allodynia.

Involvement of noradrenergic signaling in stress-induced analgesia

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It is well known that acute exposure to physical stress produces a transient antinociceptive effect (called stress-induced analgesia [SIA]). One proposed mechanism for SIA involves noradrenaline (NA) in the central nervous system. NA has been reported to activate inhibitory neurons in the spinal dorsal horn (SDH), but its *in vivo* role in SIA remains unknown. In this study, we found that an antinociceptive effect on noxious heat after acute exposure to restraint stress was impaired in mice with a conditional knockout of α_{1A}-adrenaline receptors (α_{1A}-ARs) in inhibitory neurons (*Vgat-Cre*;*Adra1a*$^{flox/flox}$ mice). A similar reduction was also observed in mice treated with DSP-4, a selective neurotoxin for NAergic neurons in the locus coeruleus (LC). Furthermore, whole-cell patch-clamp recordings using spinal cord slices revealed that NA-induced increase in the frequency of spontaneous inhibitory postsynaptic currents in the substantia gelatinosa neurons was suppressed by silodosin, an α_{1A}-AR antagonist, and by conditional knockout of α_{1A}-ARs in inhibitory neurons. Moreover, under unstressed conditions, the antinociceptive effects of intrathecal NA and phenylephrine on noxious heat were lost in *Vgat-Cre*;*Adra1a*$^{flox/flox}$ mice. Our findings suggest that activation of α_{1A}-ARs in SDH inhibitory neurons, presumably via LC-NAergic neurons, is necessary for SIA to noxious heat.

Cellular stress-induced formation of RNA G-quadruplexes accelerates α-Synuclein aggregation

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Synucleinopathies are neurodegenerative diseases caused by aggregation of α-Synuclein (α-Syn). While it has been suggested that pathogenic α-Syn can spread in the whole brain like a prion protein, those molecular mechanisms are still unknown. Here, we show that RNA G-quadruplexes (G4RNAs) have an important role in α-Syn aggregation. We found that α-Syn binds to guanine-enriched RNA sequences using RNA Bind-n-seq *in vitro*. In addition, α-Syn preferentially formed a complex with G4RNAs than with other RNA secondary structures. Under the molecular crowding conditions, α-Syn underwent liquid-liquid phase separation (LLPS), and G4RNAs significantly facilitated liquid-to-solid transition of α-Syn. In α-Syn overexpressing cells, α-Syn preformed fibril (PFF) increased G4RNA foci and in turn formed α-Syn aggregates. Furthermore, α-Syn aggregates were colocalized with G4RNA foci in the dopaminergic neurons of α-Syn PFF-injected mice. These observations suggest that G4RNA is a key factor of α-Syn aggregation and cell-to-cell transmission under the pathological condition. We are trying to reveal the mechanisms underlying increases of G4RNA foci by cellular stress, and define endogenous G4RNA forming RNAs involved in α-Syn phase transition.

Therapeutic targeting expanded DNA using cyclic pyrrole-imidazole polyamide in CAG/CTG triplet repeat neurological diseases.

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Expanded CAG/CTG triplet repeats are causal in a number of human neurological disorders, and can be classified into two types according to the location of the repeats; 1) The CAG repeat expansion in coding regions, including Huntington's disease (HD), spinocerebellar ataxia (SCA) type-1, 2, 3, 6, 7, and 17, spinal and bulbar muscular atrophy (SBMA), and dentatorubral pallidoluysian atrophy (DRPLA). 2) The CAG/CTG repeat expansion in noncoding, especially in 3' untranslated regions (3'-UTRs), including myotonic dystrophy type 1 (DM1) and SCA8. Here, we show a DNA targeting compound, cyclic Pyrrole-Imidazole Polyamide (cPIP) can suppress the pathogenesis of coding and noncoding CAG/CTG repeat expansion diseases. cPIP bound to duplex as well as hairpin CAG/CTG DNA specifically, inhibiting the RNA polymerase II passage in a repeat length dependent manner in vitro. cPIP inhibits the CAG/CTG repeat-derived mRNA transcript, result in reduction of pathogenic CUG-RNA foci and polyglutamine (polyQ) accumulations. This study presents a candidate compound for targeting pathogenic expanded CAG/CTG repeat DNA, demonstrating the concept of lowering levels of repeat disease-causing RNAs and proteins.

Regulation of function and metabolism of amyloid-beta precursor protein by Semaphorin3A-PlexinA signaling

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The amyloid-beta peptide (Aβ), a major component of senile plaques, is believed to be the underlying trigger of the development of Alzheimer's disease (AD). The elucidation of mechanism(s) that induce Aβ overproduction from its type I transmembrane precursor protein (APP) is therefore important for the development of effective AD therapies. Semaphorin3A (Sema3A), a secreted type of repulsive axon guidance molecule, is implicated in the development of various neurodegenerative diseases. It was reported that Sema3A and its signaling molecules accumulated and aggregated in the brain of AD patients. However, the molecular link between Sema3A signaling and AD pathogenesis remains unknown. Here, we provide evidence regarding the interaction between APP and PlexinA, a Sema3A receptor component, through their extracellular regions. We also narrowed down the interacting regions to 100 amino acid or less. Based on these findings, we are now investigating whether the APP-PlexinA interaction affects APP function and metabolism, which might provide novel insights into involvement of Sema3A signaling in overproduction of Aβ.

Changes in the expression of fatty acid-binding protein subtype 3 (FABP3) in the median eminence of mouse brain with pain.

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Aims: We have already shown that the alteration of polyunsaturated fatty acid (PUFA) in the hypothalamus is involved in the regulation of pain. However, how become the changes in composition of hypothalamic PUFA under pain state remain unclear. It is reported that three fatty acid-binding protein (FABP) subtypes, which regulate PUFA intracellular trafficking and signal transduction, are expressed in the mammalian brain. In this study, we confirmed the changes in the expression of FABP3 in the median eminence, which is part of the hypothalamus, of postoperative pain model mice.

Methods: Paw incision-induced postoperative methods were adopted as a pain model in male ddY mice. Mechanical hypersensitivity was examined by the von Frey test. The mRNA analysis of FABP subtypes were measured by real-time PCR, and cellular localization of its protein level were measured by immunofluorescent study.

Results: Postoperative pain mice elicited mechanical allodynia on day 2 after paw incision, and mRNA expression of FABP3 significantly increased in the hypothalamus of the postoperative pain model mice compared to that in control mice. FABP3 positive cells in the median eminence colocalized with Iba-1 positive cells, which is a microglial cell marker, but not neuron and astrocyte marker. Its protein level significantly increased in the median eminence on day 2 after paw incision and returned to the control level on day 4 after paw incision.

Conclusions: Our results suggest that FABP3 in the median eminence may change in pain stimuli and may be a key molecule to control pain signaling.

TAFIa/carboxypeptidase B generated by the thrombomodulin/thrombin system prevents oxaliplatin-induced peripheral neuropathy through inactivation of complement component C5a

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Prevention of oxaliplatin-induced peripheral neuropathy (OIPN) by thrombomodulin alfa (TMα) involves thrombin-dependent activation of thrombin-activatable fibrinolysis inhibitor (TAFI) and protein C (PC), in addition to inactivation of high mobility group box 1 (HMGB1). We thus analyzed the anti-OIPN effects of activated forms of TAFI (TAFIa), known as carboxypeptidase B (CPB), and PC (APC) in mice. OIPN was inhibited by TMα, an anti-HMGB1-neutralizing antibody (HAb) or TAFIa/CPB, and partially by APC. Combination of HAb with APC, but not TAFIa/CPB, at subeffective doses abolished OIPN. TAFIa/CPB at a subeffective dose in combination with APC at a maximal dose also abolished OIPN. Intraplantar (i.pl.) HMGB1-induced allodynia was inhibited by TMα, but not APC or TAFIa/CPB. TAFIa/CPB abolished the allodynia following i.pl. C5a, a complement component, and bradykinin, known to be degraded by TAFIa/CPB. The C5a-induced allodynia was also inhibited by HAb as well as a C5aR antagonist. The C5aR antagonist, but not combination of B1 and B2 antagonists, abolished OIPN. Our study ascertains that thrombin-dependent degradation of HMGB1 and generation of APC and TAFIa/CPB by TMα are necessary to abolish OIPN, and provides novel evidence that C5a targeted by TAFIa/CPB contributes to the development of OIPN via HMGB1-dependent mechanisms.

1-SS-31 Student Sessions

Simvastatin attenuates cardiac remodeling via Hsp90 inhibition.

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Hsp90 is a molecular chaperone that contributes to the activation and stabilization of client proteins. In our previous studies, we found that an inhibition of Hsp90 reduced cardiac remodeling during the development of heart failure in rodents. Simvastatin, an antihyperlipidemic drug, was shown to inhibit Hsp90. However, it is still unclear whether simvastatin attenuates cardiac remodeling via inhibition of Hsp90. Therefore, we investigated effects of simvastatin on the development of heart failure following myocardial infarction in rat. The results showed that treatment of the animals with simvastatin attenuated the development of cardiac fibrosis. Furthermore, we found that simvastatin attenuated the interaction of Hsp90 with c-Raf and calcineurin and decreased their contents. These results suggest that Hsp90 inhibition by simvastatin treatment is, at least in part, responsible for the reduction in the development of myocardial remodeling following acute myocardial infarction.

A PRMT5 selective inhibitor EPZ015666 inhibits pressure overload-induced left ventricular dysfunction through the suppression of cardiac hypertrophy and fibrosis

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Heart failure (HF) is a principal cause of death and disability in industrialized countries. Since cardiomyocyte hypertrophy and myofibroblast differentiation are caused during the progression of HF, the suppression of these processes is considered to be therapeutic strategy. The aim of this study is to determine the effect of a PRMT5 selective inhibitor EPZ015666 (EPZ) on left ventricular dysfunction.

Primary cultured cardiomyocytes from neonatal rats were treated with EPZ and stimulated with phenylephrine (PE). PE-induced cell hypertrophy was significantly suppressed by the treatment with EPZ. During cardiomyocyte hypertrophy, various fetal gene expression is induced. PE-induced increase in the expression of hypertrophic genes was significantly inhibited by EPZ treatment. EPZ also suppressed transforming growth factor-beta (TGF-b)-induced myofibroblast differentiation in cultured cardiac fibroblasts. Next to examine the effect of EPZ on pressure overload-induced heart failure *in vivo*, we used the transverse aortic constriction (TAC) surgery in the mouse. Echocardiographic analysis showed that TAC-induced left ventricular hypertrophy and dysfunction were significantly improved by treatment with EPZ.

These data indicate that the pharmacological inhibition of PRMT5 suppresses pressure overload-induced pathological HF.

Differences in the onset of heart failure due to aging and gender: an analysis using a mouse model of heart failure

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Heart failure with preserved ejection fraction (HFpEF) is a common syndrome in the elderly population, especially in women. To elucidate the mechanism of HFpEF, we investigated the effects of aging and sex on cardiac function in a "two-hit" HFpEF model, which combines metabolic and mechanical stress (*Nature*. 568, 351-356, 2019). We induced HFpEF in 6-month-old male- and 2-year-old male- and female mice by "two-hit" model and examined the effects of aging and sex on cardiac function. Cardiac function was evaluated by echocardiography at the beginning 0, 5, and 15 weeks of the experiment. Before the treatment, aged mice showed mild cardiac hypertrophy and reduced left ventricular contractility compared to the young mice, and there was no clear sex difference in cardiac function in aged mice. At 5 weeks, young mice showed diastolic dysfunction and cardiac hypertrophy. In sharp contrast to young mice, some aged mice showed markedly reduced left ventricular contractility. Additionally, some aged mice showed symptoms of heart failure at 15 weeks, showing more varied consequences than the young mice. In particular, some mice developed heart failure with reduced ejection fraction, which was not observed in young mice. Collectively, our results demonstrate the age-related differences in the response to the "two-hit" HFpEF model.

The curcumin analog, GO-Y022 suppressed the pressure overload-induced systolic dysfunction at a lower concentration than curcumin

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Background: We previously reported that natural compound curcumin suppresses cardiomyocyte hypertrophy and the development of heart failure via inhibiting p300 histone acetyltransferase (HAT) activity. In this study, we investigated the effect of curcumin analogue, GO-Y022 on p300-HAT activity, cultured cardiomyocyte hypertrophy and heart failure in vivo.

Methods & Results: In vitro HAT assay using recombinant p300-HAT domain showed that GO-Y022 inhibited p300-HAT activity as well as curcumin. Primary cultured cardiomyocytes prepared from neonatal rats were treated with curcumin or GO-Y022 and stimulated with phenylephrine (PE). 1 μM GO-Y022 suppressed the following results to the same extent as 10 μM of curcumin: PE-induced histone acetylation, hypertrophic response gene transcription, and cardiomyocyte hypertrophy. Finally, 8-week-old C57BL/6J male mice were subjected to transverse aortic constriction (TAC) surgery and orally administrated GO-Y022 or curcumin for 8 weeks. Cardiac echography indicated that a low dose of GO-Y022 (1 mg/kg) repressed TAC-induced increase in left ventricular posterior wall dimension and decrease in Fractional shortening to the same extent as 50 mg/kg of curcumin.

Conclusion: GO-Y022 may be used for heart failure therapy at a lower dose than curcumin.

Exploration of preventive drugs for sunitinib-induced heart failure utilizing large-scale medical database

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BACKGROUND: Several studies have reported that patients treated with sunitinib, a tyrosine kinase inhibitor, have developed left ventricular dysfunction and heart failure, but there is currently no treatment for heart failure with sunitinib. The purpose of the present study is to identify candidate drugs for the treatment of sunitinib-induced heart failure using a large medical database. METHOD: We analyzed the FDA Adverse Event Reporting System (FAERS) and the WHO global adverse event reporting database (VigiBase) to find candidate drugs for prevention of sunitinib-induced heart failure. The effects of the candidate drugs on cell viability and cell morphology were evaluated using the WST-8 assay and immunostaining in H9c2 cells derived from rat cardiac rhabdomeres. RESULTS: FAERS and VigiBase searches revealed significantly higher reporting odds ratio (ROR) of heart failure in patients treated with sunitinib than in those not treated with sunitinib. The ROR was reduced by concomitant use of Vitamin D (FAERS: ROR 0.50, 95% CI 0.26-0.96; VigiBase: ROR 0.37, 95% CI 0.10-0.95). In vitro, Vitamin D significantly improved the viability and maintained the cell morphology in H9c2 cells exposed to sunitinib. CONCLUSION: The findings suggest the potential value of Vitamin D in preventing sunitinib-induced heart failure.

Carvedilol ameliorated UK14304-induced vascular relaxation response via AMPK in diabetic mice aortas

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Carvedilol, a nonselective β-adrenergic receptor blocker, has been reported to improve endothelial dysfunction in patients with cardiovascular diseases and in a diabetic animal model but that mechanism of action is unknown. The purpose of this study was to investigate the effect of carvedilol on the endothelial-response of aortas from diabetic mice and the underlying mechanism. Vascular reactions and protein expressions were measured in aortas isolated from both control and nicotinamide and streptozotocin-induced diabetic mice (DM). UK14304 (UK)-induced endothelial-dependent relaxation declined along with the decrease of nitric oxide (NO) levels in aortas from DM. Carvedilol prevented the inhibition of UK-induced relaxation and NO production caused by DM. The phosphorylation of Akt and endothelial nitric oxide synthase (eNOS) were very low in UK-stimulated DM aortas compared with those of control group. Treatment with carvedilol significantly increased not only the phosphorylation of Akt and eNOS under UK-stimulation but also the AMP-activated protein kinase (AMPK) phosphorylation in the DM. In conclusion, carvedilol significantly ameliorated the endothelial dysfunction in DM aortas, in which increased NO levels through Akt/eNOS activation, up-regulated AMPK phosphorylation may be involved.

The effect of *Eucommia ulmoides* leaf extract on aortic dissection onset in mice

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Aortic dissection is a severe aortic disease, in which the aortic wall is separated into two layers at the medial level, resulting in two lumens being created, a true and a false lumen. Most cases have a sudden onset resulting in death. Therefore, it is required to establish a preventive strategy. *Eucommia ulmoides* leaf (EUL) extract contains various flavonoids such as quercetin, chlorogenic acid, geniposidic acid, and so on, and it is suggested to have a protective effect against cardiovascular diseases. In this study, we investigated the preventive effect of EUL on the onset of aortic dissection.

We generated pharmacologically-induced aortic dissection model mice (LAB model). In C57Bl / 6J mice, three agents are administered; (1) nitric oxide synthase inhibitor (L-NAME) that causes vascular endothelial damage, (2) angiotensin II (Ang II) that causes hypertention, and (3) lysyl oxidase inhibitor (BAPN) that causes medial fragility. EUL extract was orally administered daily throughout the experiment.

Hypertension, caused by Ang II+BAPN loading was significantly suppressed by EUL. In the LAB model, macrophage infiltration into the aortic wall was increased, but it was suppressed by EUL administration. As a result, EUL showed the preventive effects against the onset of aortic aneurysm, dissection, and death from rupture.

Effects of Suramin administration on hemodynamics during myocardial ischemia induced by anaphylaxis in rats.

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[Background and Purpose] We have reported that myocardial ischemia was observed by anaphylaxis (The 94th Annual Meeting of the Pharmacological Society). In this study, we examined the effect of Suramin, an antagonist of both P2X and P2Y2 receptors, on electrocardiogram ST-segment elevation and hemodynamics during anaphylaxis induction.
[Method] Rats received Suramin 100 or 300 micro-M or physiological saline as a control 30 minutes prior to the induction of anaphylaxis following administration of compound 48/80 (C48/80). Blood pressure and electrocardiography recording were started before Suramin administration and continued until 30 minutes after anaphylaxis induction and assessment.
[Results] ST-segment elevation was observed after C48/80 administration, which was significantly suppressed by each dose of Suramin pretreatment. After induction of anaphylaxis, heart rate, systolic and diastolic blood pressure, and rate pressure product (RPP) decreased which was significantly suppressed by pretreatment with 300 micro-M of Suramin.
[Consideration] These results suggest that pretreatment with Suramin may partially improve the hemodynamic deterioration due to anaphylaxis.

Assessment of BMS-986094-induced chronic cardiotoxicity using human iPS cells-derived cardiomyocytes

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【Introduction】 Evaluation of drug-induced cardiotoxicity is important to avoid adverse effects, such as arrhythmia and contractile dysfunction, which is considered to result in heart failure, in non-clinical and clinical studies. For example, BMS-986094, which was developed as a Hepatitis C virus nucleotide polymerase (non-structural 5B) inhibitor, was withdrawn from phase 2 clinical trials because of unexpected heart failure by long-term administration. Although animal models have been widely used to assess cardiac contraction, *in vitro* models are expected to assess human-specific contraction. We have previously developed the methods to assess cardiac contraction using motion analyses in human iPS cell-derived cardiomyocytes (hiPSC-CMs). Here we assessed whether BMS-986094 induced chronic contractile dysfunction in hiPSC-CMs.

【Methods】 We used iCell cardiomyocyte 2.0 (CDI). Motion analyses were performed using a cell motion imaging system (SI8000, Sony). Calcium imaging was performed using Fluo-8/AM.

【Results】 We found that BMS-986094 decreased both contraction and relaxation velocity by 96 h exposure, while acute exposure with BMS-986094 had little effects. In contrast, sofosbuvir, which has the same drug target, had little effect during 6-day exposure. Next, we analyzed the effect on calcium transient. BMS-986094 decreased calcium transient at 96 h, while sofosbuvir did not affect.

【Conclusion】 Thus, the imaging analysis of hiPSC-CMs would be useful to assess the chronic contractile dysfunction in human.

Effects of Tanshinone VI on the differentiation from cardiac stem cells into cardiomyocytes

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Tanshinone VI (TanVI) is prepared from *Salvia miltiorrhiza* Bunge, which has been widely used for treatment of cardiovascular disease in Chinese medicine. In our previous study, we showed the differentiation effect of TanVI on cardiosphere-derived cell (CDC) prepared from adult rat cardiac tissue. However, the exact mechanism of TanVI underlying the differentiation from cardiac stem cells to cardiomyocytes is yet unclear. In this study, we examined the effect of TanVI on the intracellular signaling pathway in CDCs during myocardial differentiation. After CDCs in the presence of TanVI were cultured, the cells expressed a cardiomyocyte marker cardiac troponin T. Concomitantly, an increase in the expression of the cardiac transcription factor Nkx2.5 was also observed in TanVI-treated CDCs. Treatment of the cells with TanVI resulted in reduction of GSK3β phosphorylation. The β-catenin expression level in CDCs was also decreased. Furthermore, TanVI reduced the phosphorylation levels of Erk1/2. These results suggest that TanVI enhances the differentiation of CDCs into cardiomyocytes via an attenuation of GSK3β, β-catenin and Erk1/2 phosphorylation.

Elucidation of the onset mechanism of exercise-induced acute kidney injury using renal hypouricemia type-1 model mice and the effects of xanthine oxidoreductase inhibitor

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Hereditary renal hypouricemia type-1 (RHUC1) is a rare disease associated with markedly lower plasma urate (UA) and increased fractional excretion of UA (FE_{UA}) due to URAT1 dysfunction, and exercise-induced acute kidney injury (EIAKI) is known to be a serious complication, but its pathogenesis is unknown. The aim of this study is the investigation of pathogenesis of EIAKI and the effect of topiroxostat, a non-purine-type xanthine oxidoreductase inhibitor (XOI) using high HPRT activity *Urat1-Uox* double knockout (DKO) mice establishing as novel animal model of RHUC1. DKO mice were used in a forced swimming test as loading exercise to explore the onset mechanism of EIAKI and evaluate related purine metabolism and renal injury parameters. In DKO mice, exercise exacerbated renal injury and functional markers, and increased urinary UA/Cr ratio and plasma UA. In addition, NLPR3 inflammasome activation and increased IL-1β were observed in the kidney. Finally, we demonstrated that topiroxostat improved renal injury and functional parameters of EIAKI. The pathogenic mechanism of EIAKI was found to be due to increased levels of IL-1β via NLRP3 inflammasome signaling associated with excessive urinary UA excretion. In addition, topiroxostat, a non-purine-type XOI, appears to be a promising therapeutic agent for the treatment of EIAKI.

Histamine H3 receptor expressed on pancreatic β-cells downregulate its insulin secretion and proliferation

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Histamine receptor H_3 (H_3R), a $G_{i/o}$-coupled receptor, is dominantly expressed in the central nervous system and regulates neurotransmitter release. Our previous study showed that H_3R was also expressed in the pancreatic β cells and had an inhibitory effect on glucose-induced insulin secretion from MIN6 cells, a cell line from mouse pancreatic β cells. However, the *in vivo* roles of H_3R on β cells in the regulation of plasma insulin level are still unknown. In the present study, we generated and phenotyped β cell specific H_3R knockout mice (cKO mice) to elucidate the importance of H_3R for glucose homeostasis. Blood glucose testing showed that H_3R deletion from β cells resulted in the lower glucose levels after glucose challenge due to higher insulin secretion. Glucose-induced insulin secretion from isolated cKO islets was higher than that from control islets. These data demonstrated that disruption of H_3R led to higher insulin secretion in vivo. Immunohistochemical analysis showed that the number of Ki67-positive β cells was augmented in cKO islets. Morphometric analysis revealed the increased number and size of islets in cKO pancreas. These data indicated that proliferation of β cells was enhanced by H_3R deletion. In conclusion, H_3R in pancreatic β cells has negative impact on insulin secretion and their proliferation.

1-SS-43 Student Sessions

Analysis of aging-induced neural dysfunctions and their biological basis

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Aging causes cognitive and motivational declines, but the biological basis remains elusive. Here we analyzed distinct behavioral effects of aging in C57BL6N (B6N) and C57BL/6J (B6J) strains. In this study, mice first learned a visual discrimination task to obtain food rewards by responding to the correct one of two visual stimuli. Then, they learned a response direction task of responding to either left or right for food rewards. Attentional set-shifting, behavioral flexibility between the tasks, is known to depend on working memory. Aged B6N mice showed motivational declines in both tasks. By contrast, task motivation was intact in aged B6J mice, but some of them showed a deficit in attentional set-shifting. We also analyzed synaptic proteomes in the medial prefrontal cortex, a brain region crucial for attentional set-shifting. Young and aged B6J mice showed differential expression of many synaptic proteins, some of which increased only in a subset of the aged mice with attentional set-shifting intact. These findings suggest that different biological mechanisms related to genetic and synaptic factors underlie motivation and cognitive declines with aging.

Deletion of SIRT1 in the skeletal muscle causes suppression of autophagy and muscle atrophy.

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[Background] Autophagy in the skeletal muscle maintains muscle mass. The NAD^+-dependent deacetylase SIRT1 positively regulates autophagy. Here, we examined whether SIRT1 in the skeletal muscle maintains muscle mass via promoting autophagy.
[Methods and Results] We obtained tibialis anterior muscle (TA) from 60-weeks old wild-type mice (WT) and muscle-specific SIRT1 knockout mice (SIRT1MKO). Western blotting showed that acetylated lysine levels were increased in SIRT1MKO compared with WT, suggesting suppressed SIRT1 activity in SIRT1MKO. Histological analysis by HE staining showed that the myofiber diameter was 9% smaller in MKO than WT. The percentage of central nuclei, an indicator of muscle regeneration, was 19% higher in SIRT1MKO than WT. To test autophagic activity in the muscle, we treated mice with colchicine, an inhibitor of autophagosome degradation, and measured markers of autophagosomes including LC3-II/LC3-I ratio (Western blotting) and the level of LC3 dots (Immunostaining). At baseline, LC3-II/LC3-I ratio and LC3 dot levels were unchanged in MKO. However, colchicine treatment increased the LC3-II/LC3-I ratio and the LC3 dot level in WT but not in SIRT1MKO, suggesting suppression of autophagic flux in SIRT1MKO. Levels ubiquitinated proteins, which are degraded by autophagy, was also increased in SIRT1MKO.
[Conclusion] These results suggest that SIRT1 plays a critical role in maintenance of skeletal muscle mass via positive regulation of autophagy.

Comparison of the binding characteristics of [^{18}F]SNFT-1 and other tau imaging tracers to Alzheimer's disease pathology

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Introduction: Misfolded tau aggregates associated with clinical syndromes and various neurodegenerative diseases. To date, many second-generation of tau PET tracers were developed to overcome the limitation of the first-generation such as off-target binding. Recently, we developed [^{18}F]SNFT-1 from compound optimization of [^{18}F]THK-5351, which showed high affinity for monoamine oxidase-B (MAO-B) as off-target binding. The aim of study was to compare the binding properties of [^{18}F]SNFT-1 and second-generation tau PET radiotracers to human brain tissues.
Methods: *In vitro* competitive binding assay were performed for tau aggregates, amyloid aggregates, and recombinant MAO-A and MAO-B. After preparing ^{18}F-labeled compounds, *in vitro* autoradiography was performed using frozen human brain sections with immunostaining of phosphorylated tau (tau IHC) for evaluation of binding selectivity.
Results and Discussion: Although second-generation of tau PET radiotracers showed a similar binding affinity for tau aggregates, the off-target binding affinity was different. SNFT-1 showed a high binding affinity for tau aggregates, which was comparable to MK-6240, with no interaction of amyloid aggregates as well as MAO enzymes. *In vitro* autoradiography demonstrated that distribution of radiotracer's binding was similar to tau IHC in the medial temporal area of Alzheimer's disease. On the other hands, second-generation tau PET radiotracers showed little binding to the frontal cortex in a case of progressive supranuclear palsy that contains high density tau aggregates.
Conclusion: [^{18}F]SNFT-1 would be a promising candidates for imaging tau aggregates. Further binding characterization is required to validate the binding of non-AD tau aggregates.

Examining the mechanisms underlying bladder fibrosis associated with injured intrapelvic nerves in rats

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[Aim] Lower urinary tract symptoms due to intrapelvic nerves damage are frequent complications of pelvic surgery. Previously, we reported that bilateral injury to accessory nerves (ACN), which extend from the major pelvic ganglion, resulted in bladder fibrosis and bladder dysfunction in rats within 72 h until at least 4 weeks post-surgery. Prevention of bladder fibrosis is necessary to maintain bladder function. Herein, we examined the mechanisms underlying bladder fibrosis associated with ACN injury.

[Methods] Ten-week-old male Wistar/ST rats were categorized into sham and bilateral ACN injury (BAI) groups. In the BAI group, the ACN was crushed for 1 min using reverse-action tweezers on both sides. After 4, 8, 12, 24, and 72 h, we examined bladder histology using Masson's trichrome staining and evaluated mRNA expression levels of TGF-β1 and MCP-1 using real-time PCR analysis.

[Results] Increased collagen area-to-total bladder area ratio of BAI groups was noted at 12, 24, and 72 h postoperatively, compared to sham groups. Postoperative mRNA expression levels of TGF-β1 and MCP-1 at 12, 24, and 72 h, and 4, 8, 12, and 24 h respectively, were higher in BAI groups compared to sham groups.

[Conclusion] Upregulation of MCP-1 followed by TGF-β1 may be involved in bladder fibrosis associated with ACN injury.

Efficacy of filtered bone marrow stem cell lysate for overflow urinary incontinence in model rats

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Hypothesis. Overflow urinary incontinence (OUI) often occurs as a complication after pelvic surgery. Our previous experiment found that bone marrow-derived stem cell lysate (BMSCL) improves neurogenic erectile dysfunction. In this study, we investigated the effectiveness of BMSCL in a model of OUI by bilateral accessory nerve injury (ACNI).

Methods. Rat bone marrow-derived stem cells were collected, and BMSCL (lysate of 1×10^6 cells/PBS) was prepared. Eight-week-old male Wistar-ST rats were divided into sham+PBS (sham, n=7), ACNI+PBS (ACNI, n=10), and ACNI+BMSCL (BMSCL, n=10) groups. Following surgery, PBS or BMSCL (100 μl/body) was administered intravenously. Bladder function, bladder weight, and morphology were evaluated after one week. In addition, the response of carbachol (CCh) was assessed.

Results. While 7 of 10 rats in the ACNI group showed symptoms of OUI, only 3 of 10 rats in the BMSCL group presented symptoms of OUI. Bladder weight in the ACNI group was significantly larger than in the sham group ($P<0.01$), while bladder weight in the BMSCL group was significantly lower than in the ACNI group ($P<0.01$). Fibrotic area in the ACNI group was larger than in the sham group, while that in the BMSCL group was smaller than in the ACNI group. The maximum response to CCh in bladder specimens in the ACNI group was higher than that of sham group, while this was lower in the BMSCL group than in the ACNI group.

Conclusion. BMSCL improved bladder function and morphology, suggesting that intravenous injection of BMSCL may be a useful treatment for neurogenic bladder dysfunction.

Effects of sodium valproate on cisplatin-induced acute kidney injury

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OBJECTIVE: Cisplatin-induced acute kidney injury (AKI) is well known, and the nephrotoxicity of cisplatin restricts its clinical application. Currently, there are no drugs are recommended for the prevention of cisplatin-induced AKI. Forced hydration and diuresis may partially prevent nephrotoxicity of cisplatin, but it is still difficult to entirely prevent kidney injury. Thus, establishment of a new preventive method against cisplatin-induced AKI is required. Therefore, in this study, the purpose of this study was to clarify the efficacy of sodium valproate in cisplatin-induced AKI.

METHODS: In order to establish cisplatin - induced AKI animal model, C57BL/6 mice were administered with either cisplatin (15 mg/kg, i.p.) or saline (control). The degree of renal damage was assessed by various renal function parameters and pathological evaluation. The effect of sodium valproate on cisplatin-induced cytotoxicity was evaluated using HK2 cells, MKN-1 cells and LLC cells.

RESULTS: Cisplatin treatment worsened various renal function parameters and tubular damage scores, which were significantly improved by co-treatment with sodium valproate. The decrease in cell viability of HK2 cells by cisplatin was significantly improved by co-treatment with sodium valproate. On the other hand, sodium valproate had no adverse effect on the reduction of cell viability of various cancer cells by cisplatin.

CONCLUSIONS: The results of this study indicated that sodium valproate could act as a potential preventive drug for cisplatin-induced AKI.

Mechanisms of hydrogen sulfide-induced protective effect on rat bladder dysfunction induced by cyclophosphamide

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We have reported protective effect of NaHS [hydrogen sulfide (H_2S) donor] on cyclophosphamide (CYP)-induced rat bladder dysfunction by improving CYP-induced shortening of intercontraction intervals (ICI) and increases in non-voiding contractions (NVCs). In this study, we examined mechanisms of the protective effect. Nine-week-old male Wistar rats were pretreated with NaHS (10 μmol/kg, ip) or saline once daily for 7 days. CYP (150 mg/kg, ip) or saline had been injected 2 days before urodynamic experiments, and after the experiments, bladder tissues were collected to perform HE staining. In some rats, vehicle or capsaicin (CAP, 125 mg/kg, sc), which can desensitize CAP-sensitive afferent nerves, was pretreated 4 days before urodynamic experiments. In bladder tissues, CYP increased neutrophile infiltration, bleeding, and edema, but NaHS partially improved only the edema. CAP prolonged ICI and reduced NVCs in CYP-treated rats. NaHS-induced improvement of CYP-induced ICI shortening and NVC increasing was not detected in CAP-treated rats. These data suggest that NaHS showed protective effect on bladder dysfunction in CYP-treated rats via suppression of CAP-sensitive bladder afferent nerves but not of bladder inflammation.

Contribution of LAT1 to amino acid uptake in cancer cells and suppression of cellular protein synthesis by LAT1 inhibitor

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Cancer cells require more nutrients than normal cells to maintain rapid growth and proliferation. L-type amino acid transporter 1 (LAT1: SLC7A5) is highly upregulated in various cancers, and is regarded as a molecular target for cancer therapy. LAT1 preferentially transports large neutral amino acids (Leu, Ile, Val, Met, Phe, Tyr, Trp, and His) including many essential amino acids in a Na^+-independent manner. JPH203, an LAT1-specific high-affinity inhibitor, strongly suppresses cancer cell proliferation and tumor growth. However, the contribution of LAT1 to the amino acid uptake in cancer cells and the effect of JPH203 on cellular protein synthesis have not been established. Here, we revealed that JPH203 drastically suppresses the uptake of large neutral amino acids into pancreatic cancer cell lines, regardless of the presence or absence of Na^+. This indicates that LAT1 has the main contribution to cellular amino acid uptake among amino acid transporters including Na^+-dependent transporters. Furthermore, we revealed, by polysome profiling analysis, that JPH203 treatment suppresses cellular protein synthesis. These results indicate that LAT1 constitutes the main uptake pathway for large neutral amino acids in cancer cells. Inhibition of LAT1 efficiently suppresses their uptake and reduces the protein synthesis in cancer cells.

Protein phosphatase 6 promotes neurite outgrowth by dephosphorylating SIN1

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Understanding the molecular mechanism of neuronal differentiation is extremely important to overcome the incurable diseases caused by nervous system damage. Neurite outgrowth is essential for neuronal differentiation and regeneration, and cAMP response element-binding protein (CREB) is one of the key transcriptional factors positively regulating this process. Neuronal differentiation stimuli activate mammalian target of rapamycin complex 2 (mTORC2) / Akt signaling to phosphorylate CREB. However, the molecular mechanism that regulates the activity of this signaling remains poorly understood. We found that neuronal differentiation stimuli increased a protein level of protein phosphatase 6 (PP6), a member of type 2A Ser/Thr protein phosphatases in N2a cells and mouse ES cells. The decrease in autophagy activity was suggested to be involved in the stimulation-induced increase in PP6 expression. PP6 knockdown suppressed mTORC2/Akt/CREB signaling and failed neurite outgrowth. SIN1 is a unique component of mTORC2, and dephosphorylation of SIN1 increases the activity of mTORC2 against Akt. We found PP6 knockdown increased SIN1 phosphorylation. These data suggest that PP6 may positively regulate neurite outgrowth by dephosphorylating SIN1 to activate mTORC2/Akt/CREB signaling.

Polysulfides do not mimic the sulfide-induced acceleration of $Ca_v3.2$ T-type Ca^{2+} channel activity: possible involvement of distinct affinity to zinc

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Sulfides, such as Na_2S and NaHS, enhance $Ca_v3.2$ T-type Ca^{2+} channel activity, thereby promoting pain signals. We hypothesize that, as does L-Cys, a thiol compound, sulfides might interact with zinc binding to His-191 in $Ca_v3.2$ and cancel the zinc-induced channel inhibition. On the other hand, the effects of polysulfides, such as Na_2S_3 and Na_2S_4, on $Ca_v3.2$ function have yet to be investigated. Thus, we compared the effects of sulfides and polysulfides on T-channel-dependent currents (T-currents) in human $Ca_v3.2$-expressing HEK293 cells, and analyzed possible involvement of sulfide-zinc interaction. Na_2S and NaHS at 3-30 μM rapidly caused remarkable and persistent increase in T-currents. In contrast, Na_2S_3 and Na_2S_4 in the same range caused only slight and transient T-current increase, followed slowly by its decrease below the baseline. In the presence of tricine, a weak Zn^{2+} chelator, T-currents increased, which was reversed by $ZnCl_2$ at 30 μM. The increased T-currents in the presence of tricine was not altered by Na_2S at 30 μM, but augmented by addition of Na_2S following $ZnCl_2$. Our data suggest that the sulfide-induced enhancement of $Ca_v3.2$ function involves the interaction of sulfide with Zn^{2+} possibly binding to His-191 in $Ca_v3.2$, and cannot be mimicked by polysulfides.

Cryo-EM structure of histamine H_1 receptor-G_i protein complex

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Histamine is a well-known autacoid, which widely exists in vertebrate tissues and plays various roles. Its physiological functions depend on the activation of four types of histamine-related G protein-coupled receptors (H1R, H2R, H3R, H4R). Especially, H1R is expressed ubiquitously, which is involved in most histamine-induced allergic effects.

Previous functional studies reported that H1R mainly activates G_q protein, and the following optogenetical and chemical studies revealed that inhibition of histaminergic neurons induce acute non-REM sleep. However, recent studies suggested that H1R activates four types of G protein (G_s, $G_{i/o}$, G_q, and $G_{12/13}$), and thus the signaling axis for non-REM sleep remains elusive.

Structure-guided mutgenesis is a desirable tool for extracting specific signaling, which helps the understanding of the histamine signaling mechanism. Here, we determined the histamine-bound H1R-G_i structure at 3.4 Å resolution. This structure allowed clear assignment of histamine-H1R-G_i, which reveals the signaling axis of H1R-G_i. Moreover, our structure revealed that H1R adopts the G_i-specific binding mode, which differs from the G_q-coupled H1R structure reported previously.

Our results provide a molecular mechanism for the signal switch and a molecular basis for the further development of histamine drugs.

Cryo-EM structure of the human MT_1–G_i signaling complex

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Melatonin (*N*-acetyl-5-methoxytryptamine) activates melatonin receptors (MT_1 and MT_2), which are one of the G_i-coupled class A GPCRs and transduce inhibitory signaling by inhibiting the adenylyl cyclase (AC). Melatonin thus induces our sleep and modulates our circadian rhythm, and melatonin receptors have long been regarded as an important therapeutic target for an insomnia. Although melatonin itself serves as a sleep-inducing supplement, its property is not enough to use clinically, because it is rapidly cleared from our body. Therefore, a lot of melatonin analogs with prolonged release properties have been developed so far, such as ramelteon, agomelatine, tasimelteon.

Recently reported crystal structures of ligand-bound MT_1 and MT_2 elucidated the structural basis of ligand entry and recognition, but the molecular mechanism of the ligand-induced MT_1 structural change that would lead to G_i-coupling remains unclear.

Here we report the cryo-EM structure of the MT_1-G_i signaling complex at 3.3 Å resolution. The structure reveals the receptor activation mechanism, in which the ligand-induced conformational changes are propagated to the G-protein coupling interface. As compared to other G_i-coupled receptors, MT1 exhibits a large outward movement of TM6, which is considered to be a specific feature of G_s-coupled receptors. The structural comparison among the G_i- and G_s-complexes demonstrated the conformational diversity of the C-terminal entry of the G_i protein, suggesting the loose and variable interactions at the helix end. These notions, together with our biochemical and computational analyses, highlight the different binding modes of $G\alpha i$ and provide the basis for the selectivity of G-protein signaling.

The protein level of tumor-promoting factor SET is regulated of cell density

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SET is a multifunctional protein that acts as an intrinsic inhibitor of the tumor suppressor protein phosphatase 2A and a histone chaperone. Increased SET levels have been observed in various cancers; however, the underlying molecular mechanisms remain unclear. We found that SET protein accumulates with the increasing density of cultured cells. In this study, we aimed to clarify the mechanism underlying the accumulation of SET.

High cell density increased the SET levels in all the adherent cells analyzed. This phenomenon was observed in both cancer cell lines and non-cancer cell lines. The mRNA levels of SET were not affected by cell density, while the half-life of SET was extended at high cell densities. Autophagy inhibition led to SET accumulation, indicating the involvement of autophagy. However, cell density does not affect global autophagy activity, suggesting the involvement of selective autophagy in SET degradation. SETBP1 directly binds to SET and protects it from cleavage by proteases. We found that high cell density increased SETBP1 mRNA and protein. Furthermore, altering the expression of SETBP1 suppressed the change in SET with cell density. Our data revealed a mechanism underlying the regulation of SET level, wherein increased cell density induces SETBP1 expression and protects SET from selective autophagy.

Allosteric regulation of PI3K by methyl vinyl ketone

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Methyl vinyl ketone (MVK) is an α,β-unsaturated carbonyl compound contained in smoke and exhaust gas. This compound is known to covalently bind to proteins via Michael reaction. However, the target proteins are fully unsolved yet. We found that MVK suppresses phosphatidylinositol 3-kinase (PI3K) –Akt signaling. This pathway is essential for various biological regulation, including cell survival, glucose metabolism, and autophagy. In this study, we tried to clarify the mechanism by which MVK interferes with PI3K–Akt signaling.

Initially, we investigated the effects of MVK on the phosphorylation of epidermal growth factor receptor (EGFR), PI3K and Akt by treatment with EGF in A549 cells. MVK significantly attenuated the levels of pAkt and pPI3K, but not pEGFR formation. In addition, co-immunoprecipitation analysis revealed that MVK inhibits the interaction of PI3K with EGFR. Interestingly, exposure to MVK did not change the levels of phosphatidylinositol 3,4,5-trisphosphate indicating that the catalytic domain in PI3K is not a target of MVK. Next, we employed LC-MS/MS analysis to determine the modification sites in PI3K. We identified that both Cys146 and 656 residues in PI3K p85 subunit are modified with MVK. To confirm if these sites are essential for the EGF signaling, we substituted each Cys residue with Ser. We found that EGF treatment fails to activate the phosphorylation of PI3K p85 C656S mutant but not the C146S mutant. These results indicated that MVK may be a novel type of PI3K inhibitor via modification of p85 subunit.

Regulation of Gene Expression via Protein Oxidation

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Epigenetic dysregulation, such as aberrant DNA methylation, is one of the hallmarks of cancer cells, however the mechanisms by which these dysregulations occur remain unclear. Previously, we found that DNA methyltransferase 3B (DNMT3B), which catalyzes cytosine methylation, is selectively oxidized by several stresses. A cysteine residue is the modification site on DNMT3B. As oxidation of DNMT3B attenuates its enzymatic activity, this reaction may be related to the epigenetic regulation. In this study, we focused on the cell cycle regulator *CCND2* and examined whether its expression is regulated by oxidative stress and enzymatic activity of DNMT3B. Indeed, exposure to the stress and the universal DNMT inhibitor 5-aza-2'-deoxycytidine induced *CCND2* expression. When DNMT1, DNMT3A, and DNMT3B were overexpressed, only DNMT3B significantly reduced *CCND2* mRNA levels. CS mutant, which lost enzymatic activity, had no effect on *CCND2* mRNA levels compared with the wild type. The siRNA knockdown of DNMT3B markedly enhanced *CCND2* mRNA levels. Our data strongly suggest DNMT3B-specific regulation of *CCND2* expression. Finally, bisulfite sequencing analysis revealed that oxidative stress significantly decreased DNA methylation levels in the *CCND2* promoter region. These results indicate that oxidation of DNMT3B plays an important role in epigenetic regulation and may lead us to know the mechanism of epigenetic dysregulation in cancer cells.

Differentiation-inducing factor 1 induces mitochondrial fission via Cofilin activation in endothelial cells

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Differentiation-inducing factor 1 (DIF-1) is a polyketide produced by slime mold *Dictyostelium discoidium* which inhibits growth and migration and promotes differentiation of *Dictyostelium* cells by localizing to mitochondria. DIF-1 regulates phosphorylation of signaling molecules involved in rearrangement of actin cytoskeleton and induces transient cortical accumulation of F-actin in *Dictyostelium* cells. We recently reported that DIF-1 inhibits growth and migration of various types of mammalian cell lines through, at least in part, activation of AMP-activated kinase (AMPK). However, the molecular mechanism for the effect of DIF-1 on actin dynamics remains elusive. Here, we found that DIF-1 regulates actin cytoskeleton in endothelial cells by activating Cofilin, an actin depolymerization factor. In mouse immortalized endothelial cells (SVECs), DIF-1 rapidly induced dephosphorylation and activation of cofilin, followed by actin fiber depolymerization. When Cofilin is activated, mitochondrial fission is induced. Consistently, DIF-1 induced mitochondrial fission and knockdown of Cofilin suppressed mitochondrial fission by DIF-1. These results suggested that DIF-1 regulates actin cytoskeleton and induces mitochondrial fission via Cofilin activation.

The effect of intratracheal administration of thyroid hormone (T_3) in chronic obstructive pulmonary disease (COPD) mouse model simulating disease type classification.

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【Purpose】

COPD is a refractory pulmonary disease characterized by bronchitis, emphysema and mucus stasis. Because COPD is a multifactorial disease and represents various symptoms for each patient, previous studies which regard COPD as a single disease have limits. Therefore, to establish personalized medicine for COPD we need research developments based on phenotype or endotype classification. Here, I focused on T_3 which has long been known to act as an essential factor for development and growth in the lung. This study aims to elucidate the pathophysiological role of T_3 using COPD mouse model simulating disease type classification (emphysema- or airway-dominant).

【Methods & results】

In this study, I chose elastase-induced model and C57BL/6-βENaC-Tg mice as mouse models of emphysema- and airway-dominant COPD, respectively. I intratracheally administered T_3 (40 or 80 μg/kg, every other day) to elastase-induced mice for 21 days or C57BL/6-βENaC-Tg mice for 12 days. In elastase-induced model, T_3 treatment improved emphysema and, partially, the respiratory function with upregulation of the expression of *Ppargc1a* (the master regulator of mitochondrial biogenesis) and *Gclm* (an oxidative stress-related factor) after one T_3 injection. On the other hand, in C57BL/6-βENaC-Tg mice T_3 treatment did not improve COPD pathology.

【Discussion】

These results emphasize the importance of research developments based on phenotype or endotype classification considering that T_3 effect is different between two COPD models.

Influence of fluoro-loxoprofen on gastrointestinal mucosa

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Fluoro-loxoprofen (F-LOX), which is a derivative of loxoprofen (LOX), has been reported to be less ulcerogenic response to the gastric mucosa, but its effect on the small intestinal mucosa has not been investigated. In this study, we investigated the influence of the novel anti-inflammatory drug F-LOX on the small intestine in rats.

F-LOX and LOX were orally administered to male SD rats, and the total area of macroscopic injury in the small intestine was determined 24 h later. The prostaglandin E2 (PGE2) production and cAMP contents in the small intestine were examined 3 or 6 h after administration of drugs by enzyme immunoassay.

LOX caused hemorrhagic injury along the small intestine, mainly in the jejunum and ileum 24 h later. The intestinal lesion was also observed in the F-LOX group, but it was less, and the severity was lower than in the LOX group. The PGE2 production in the small intestine was significantly decreased 3 h after LOX and F-LOX administration. The cAMP contents were significantly reduced in the LOX group but not in F-LOX group. A decrease in PAS-positive mucus was observed in the F-LOX group, but the amount of mucus was higher than that in the LOX group. In conclusion, F-LOX is an NSAID that has the same anti-inflammatory effect as LOX but is less damaging to the small intestinal mucosa.

Alleviative effect of glutamate on 5-fluorouracil-induced intestinal mucositis in mice

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Although an antitumor drug 5-fluorouracil (5-FU) frequently causes intestinal mucositis accompanied by severe diarrhea, the useful prevention and treatment have not been established. Glutamate is known to play an important role in energy metabolism in gastrointestinal tracts. The aim of this study was to investigate the alleviative effect of 5-FU-induced mucositis in mice. Intestinal mucositis was induced in male C57BL/6 mice by repeated administration of 5-FU for 6 days. Glutamate was administered orally starting from 5 days before the onset of 5-FU treatment. Disease severity was assessed by body weight and stool consistency, and the intestinal mucositis was examined histologically. The effect of glutamate on 5-FU-induced cell injury was also examined in rat intestinal epithelial cell line IEC6.
Repeated administration of 5-FU produced severe intestinal mucositis, histologically characterized by the shortening of villi and destruction of crypts, accompanied by body weight loss and diarrhea. Daily administration of glutamate significantly reduced the severity of histological intestinal injury despite little preventive effect on diarrhea and body weight loss. The pretreatment with glutamate significantly increased epithelial electrical resistance in IEC6 cells. These results suggest that glutamate prevents 5-FU-induced intestinal mucositis via enhancement of intestinal barrier functions. Thus, glutamate administration may be useful for prevention and treatment of intestinal mucositis during cancer chemotherapy.

Lansoprazole suppresses cisplatin-induced ototoxicity via inhibiting organic cation transporter 2

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Cisplatin-induced ototoxicity (CIO) is caused by cisplatin accumulation in the inner ear cochlea, which is mediated by organic cation transporter 2 (OCT2). Proton pump inhibitors, including lansoprazole (LPZ), ameliorated cisplatin-induced nephrotoxicity via inhibiting OCT2. In the present study, we investigated the protective effect of LPZ against CIO using zebrafish and real-world data. Using zebrafish, we compared the effect of LPZ on CIO through in vivo fluorescence imaging of the hair cells stained with fluorescence dyes. Cisplatin treatment to zebrafish significantly decreased the fluorescence intensities (FI) of hair cells (approximately 50% of those of control zebrafish). Co-treatment of LPZ or knockout (KO) of oct2 significantly suppressed the reduction of FI by cisplatin (approximately 75% of those of control zebrafish). The protective effect of LPZ were not significantly different between wild type and oct2-KO zebrafish. Using electronic medical records in Mie University Hospital, we validated the preventive effect of LPZ against CIO in 289 patients who received cisplatin contained chemotherapy. The rate of co-administrated LPZ in patients without ototoxicity was significantly higher than that of patients with ototoxicity (34% vs 7%). These results suggest that LPZ should suppress CIO through the inhibition of OCT2.

1-SS-63 Student Sessions

Changes in macrophage functions caused by cancer cells

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Cancers evade immunity by multiple mechanisms. Myeloid-derived suppressor cells (MDSCs) have been shown to be involved in immunosuppression in cancer patients. They are suggested to be increased and activated by tumor microenvironment. Subpopulations of MDSCs have been shown to be monocytes/macrophage-lineage cell. In addition, subpopulations of tumor-associated macrophages have also been shown to suppress inflammation and immune response in tumors, suggesting that tumor microenvironment may educate monocytes/macrophages to acquire the immune regulatory function. To test this hypothesis, we first injected a mixture of bone marrow-derived macrophages (BMDMs) and LL/2 lung cancer cells subcutaneously into mice. We found that BMDMs increased tumor growth. To further analyze, we cocultured BMDMs with LL/2 cells. Interestingly, coculturing with LL/2 dramatically increased expression of *Cd274,* encoding an immune checkpoint protein PD-L1. They also increased *Vegf* (vascular endothelial growth factor) expression. These results suggest that LL/2-educated BMDMs may promote tumor growth. Indeed, LL/2-educated BMDMs suppressed activation of $CD8^+$T cells, indicating they acquired immune suppressive activity. Our data support the notion that tumor microenvironment educate macrophages so that they support tumor survival and growth.

Potential therapeutic effects of ozone water on pyoderma and atopic dermatitis in dogs

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Ozone water is currently utilized for antibacterial and antiviral purposes under the pandemic of COVID-19 due to its strong oxidation effect without safety problems. Therefore, clinical applications of ozone water for cutaneous diseases such as atopic dermatitis (AD) and pyoderma are expected recently. The aim of this project is to apply the ozone water for AD and pyoderma in dogs. We here examined the antibacterial effects of ozone water using staphylococci in *in vitro* setting, and anti-allergic effects of ozone water in AD mouse model.

We first examined the bactericidal effect of ozone water on resident staphylococci on skin. *Staphylococcus aureus*, which is an exacerbating factor in human AD, and *S. lentus* collected from AD mouse model was treated with ozone water and the number of bacteria was determined. Our findings indicated that ozone water showed a significant bactericidal effect against both staphylococcal species . We are currently investigating the efficacy of ozone water against staphylococci isolated from AD and pyoderma dogs. *In vivo* experiment with AD mouse model is also underway to look for the therapeutic effects of ozone water with impact on inflammatory and itch responses.

Development of monoclonal antibody-based enzyme immunoassay for tetranor-Prostaglandin D Metabolite

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Tetranor-Prostaglandin D Metabolite (tetranor-PGDM) is a metabolite of PGD_2. Urinary tetranor-PGDM level is increased in some diseases, including food allergy. In this study, we developed a monoclonal antibody (MAb) and a competitive enzyme immunoassay (EIA) for measuring tetranor-PGDM. Spleen cells isolated from mice immunized with tetranor-PGDM were utilized to generate Ab-producing hybridomas. We chose hybridomas and purified MAb against tetranor-PGDM to develop competitive EIA. The assay evaluated the optimal ionic strength and pH. Specificity was determined by cross-reactivity to tetranor-PGEM, tetranor-PGFM, and tetranor-PGAM. Recovery was determined by spiking experiments on artificial urine. Optimal ionic strength was 150 mM NaCl, and optimal pH was pH 7.5. Metabolites other than tetranor-PGDM did not show any significant cross-reactivity in the EIA. The assay exhibited range of quantitation (ROQ) value of 0.252 to 20.2 ng/mL. The linearity-dilution effect showed excellent linearity under dilution when artificial urine samples were applied to solid-phase extraction (SPE). After SPE, recovery of tetranor-PGDM in artificial urine averaged from 82.3% to 113.5% and was within acceptable limits (80%–120%). We successfully generated one monoclonal antibody and developed a sensitive competitive EIA.

The Effect of Combined Bcr-Abl Inhibitor and ALDH Inhibitor on Chronic Myelogenous Leukemia

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【Introduction】Chronic myelogenous leukemia (CML) has become a disease with five-year-survival rate of more than 90% according to appearance of Bcr-Abl inhibitor. Stopping treatment with Bcr-Abl inhibitors leads to relapse in many patients. This is due to the fact that Bcr-Abl inhibitors are not effective against cancer stem cells, and suggests that a different drug is necessary to eradicate cancer stem cells. ALDH is overexpressed in cancer stem cells and promotes their survival. We have shown in past studies that Bcr-Abl inhibitors do not inhibit ALDH expression. In this study, we examine the effect of ALDH inhibitors on CML.

【Methods】We examined the effects of an ALDH inhibitor using K562 cells, which are CML cell line. We verified the effect of an ALDH inhibitor against CML using a WST-8 assay. We then measured ALDH protein expression using flow cytometry.

【Results】ALDH inhibitors reduced cell survival in a concentration-dependent manner, and when combined with a Bcr-Abl inhibitor, cell viability was synergistically decreased. ALDH protein expression was decreased in cells treated with ALDH inhibitor alone or Bcr-Abl inhibitor alone according to flow cytometry. In cells treated with ALDH inhibitor and Bcr-Abl inhibitor, ALDH protein expression was more strongly repressed.

【Conclusion】The results of this study suggest that ALDH inhibitor use might be effective in CML treatment.