1-SS-00 Student Sessions

Establishment of chronic postsurgical pain model and analysis of its therapeutic target

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Involvement of supraspinal orexin-A/orexin receptors in the regulation of central post-stroke pain

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Central post-stroke pain (CPSP) is one of the secondary diseases of cerebral stroke. However, the detailed mechanism remains unclear. Recently, it is reported that the ablation of supraspinal orexin neurons induced mechanical allodynia in mice. In this study, we tested the involvement of supraspinal orexin system in the regulation of CPSP. Male ddY mice (5 weeks old) were subjected to 30 min of bilateral carotid artery occlusion (BCAO). Colocalization of fluorogold (a retrogradely transported tracer) with orexin-A were determined by double-immunofluorescence. Mechanical allodynia was measured by von Frey filament test. Intrathecal (i.t.) injection of fluorogold was colocalized with orexin-A positive cells in the hypothalamus. On day 3 after BCAO, the withdrawal responses to mechanical stimuli were significantly increased and prepro-orexin was decreased as compared with sham. The BCAO-induced mechanical allodynia suppressed by the intracerebroventricular injection of orexin-A. This effect of orexin-A was significantly inhibited by the i.t. injection of an orexin 1 or 2 receptor antagonist. These results suggest that the supraspinal orexin-A system mediated by the OX1R and OX2R play an important role in the regulation of CPSP.

1-SS-02 Student Sessions

Identification of a binding protein for 2ccPA and characterization of its roles in microglial cell death

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Cyclic phosphatidic acid (cPA) is a naturally occurring phospholipid mediator found in mammalian tissues and cells. It has been reported that cPA has novel biological activities; attenuates traumatic brain injury-mediated neuronal cell death and inhibits chronic and acute inflammation. However, there are some seemingly unanswerable questions. In this study, our lab has successfully used Click chemistry approach to derivatize azide-tagged metabolically stabilized cPA analogue, 2-carba-cPA (2ccPA). Using affinity chromatography with 2ccPA beads, we successfully captured a potential target protein (30k) from the mouse microglial cell (SIM-A9). We then analyzed by LC-MS/MS and identified adenine nucleotide translocase 2 (ANT2) as a 2ccPA binding protein. We would like to discuss on potential roles of 2ccPA-ANT2 axis regulating microglial mediated neuroinflammation and related diseases.
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Type 1 metabotropic glutamate receptor 1 and GABA B receptor form complexes at cell membrane and mutually modulate their function

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G-protein coupled receptors (GPCRs) are one of the largest membrane protein families in eukaryotes and mediates various important function of the cells. Recently, there is increasing evidence that several GPCRs may form complexes, and complexed GPCRs may mutually regulate their function. Previous reports including ours unveiled the interaction between two GPCRs expressed in neurons, type 1 metabotropic glutamate receptor (mGluR1) and gamma-aminobutyric acid B receptor ($GABA_BR$). mGluR1 is expressed particularly in cerebellum and known to play crucial roles in synaptic plasticity and motor learning. $GABA_BR$ is widely expressed in central nervous system and regulates neuronal excitability. Our previous study suggested that $GABA_BR$ modulates mGluR1-mediated synaptic plasticity in cerebellar Purkinje cell. In this study, we showed the mechanism underlying modulation between mGluR1 and $GABA_BR$. Biochemical analysis and molecular imaging assay showed that mGluR1 form complexes with $GABA_BR$ at the cell membrane. Moreover, an assay monitored intracellular signaling transduction revealed functional interaction between these GPCRs. Our findings provide a novel insight into the regulatory mechanism of synaptic plasticity and motor learning, and indicate that complex formation and functional interaction between distinct GPCRs might have significant roles under physiological and pathophysiological conditions.

Characterization of direction-specific vagus nerve spikes in a freely moving rodent

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The vagus nerve is a parasympathetic nerve and plays a critical role in the regulation of autonomic functions to maintain homeostasis including heart rate, respiratory rhythms and gastric motility. It remains largely unknown about vagal physiological functions in naïve behavior due to technical limitations of vagal spike recordings. Here, we developed a vagal spike recording technique with a cuff-shaped electrode in a freely moving rodent animal while simultaneously monitoring central and peripheral bioelectrical signals. The spike rates of both afferent and efferent fiber types increased simultaneously following increased locomotion and were higher in novel environment than in familiar one. The electrode contains multiple recording sites to identify afferent and efferent vagal spikes. For manipulating vagus nerve spikes, we used two strains of transgenic mice expressing channelrhodopsin2, a photosensitive protein, in afferent and efferent vagal fibers. These results provide novel insights into vagal physiological function and make a new step forward to uncover a neurophysiological basis underpinning the brain-body axis.

OX2R-selective orexin agonism is sufficient to ameliorate narcoleptic symptoms, cataplexy and sleep/wakefulness fragmentation in mouse models

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Loss of orexin-producing neurons in the lateral hypothalamus causes the chronic sleep disorder narcolepsy-cataplexy. Narcoleptic humans suffer from two major symptoms, excessive sleepiness and cataplexy in the active phase, and these symptoms in mouse models are manifested as sleep/wakefulness fragmentation and SOREMs (direct transitions from wakefulness to REM sleep), respectively. The neuropeptides orexin-A (OXA) and orexin-B (OXB) act on two receptors orexin type-1 receptor (OX1R) and orexin type-2 receptor (OX2R). Orexin receptor agonists are expected to be of potential value for treating human narcolepsy. Here, to confirm the fundamental strategy aimed at improving narcoleptic symptoms, we examined the association between orexin receptor subtypes and these symptoms by intracerebroventricular (ICV) administration of the OX2R-selective agonist [Ala^{11}, $_{D\text{-}}Leu^{15}$]-OXB in orexin knockout mice. OXA and [Ala^{11}, $_{D\text{-}}Leu^{15}$]-OXB similarly decreased the number of SOREMs. Further, transition frequencies between NREM sleep and wake states in narcoleptic model mice were similarly decreased. We confirmed in vivo that [Ala^{11}, $_{D\text{-}}Leu^{15}$]-OXB did not activate OX1R-expressing LC noradrenergic neurons by Fos staining. Therefore, OX2R-selective agonism is sufficient to ameliorate narcoleptic symptoms, both cataplexy and fragmentation of wakefulness in model mice. Activation of LC noradrenaline neurons expressing OX1R are not essential for suppression of these symptoms.

ERK1/2-containing MPs from diabetic mice induce endothelial dysfunction via the vascular ERK1/2 activity.

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Microparticles (MPs) which are micro vesicles shed from the membrane of vascular and blood cells are considered as one of the causes of endothelial dysfunction in the diabetic vascular complications. In this study, we examined the effect of MPs derived from diabetes mice on vascular function, focusing on extracellular signal-regulated kinases (ERK)1/2-conteining MPs. MPs were prepared from streptozotocin (STZ)-induced diabetic mice (STZ), controls (Cont) and STZ and Cont mice treated with ERK1/2 inhibitor, PD98059 (PD). Vascular reactions and protein expressions were examined. STZ-derived MPs (STZ MPs) were found to have increased amounts of MP and to be attached to the endothelial cells as compared to Cont-derived MPs (Cont MPs). Furthermore, we found that ERK1/2 was contained in the MPs, especially STZ MPs. In addition, ERK1/2 activity and expression were increased in Cont vessels treated with STZ MPs. STZ PDMPs (PD-treated STZ derived MPs) improved the attenuated endothelial dependent relaxation in aortic rings. On the other hand, direct treatment of PD in STZ aortic rings did not improve the attenuated endothelial dependent relaxation. These results suggested that ERK1/2-containing MPs regulate ERK1/2 activity in blood vessels and cause endothelial dysfunction during diabetes.

Influence of long-term exposure of advanced glycation endproducts on vascular contraction in rat carotid artery

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Although there are several reports suggested that advanced glycation end-products (AGEs) cause vascular endothelial dysfunction, the direct relationship between AGEs and smooth muscle contractile function remains unclear. Therefore, we investigated the long-term effects of AGE-bovine serum albumin (AGE-BSA) on contractile responses in rat carotid arterial rings using organ-culture technique. After exposure of AGE-BSA (0.001-0.1 mg/mL) for approximately 1 day in carotid artery, concentration–response curves were investigated under endothelium denuded artery. Contractile responses of high K^+ or serotonin did not alter among groups treated with and without AGE-BSA. Treatment with AGE-BSA (0.1 mg/mL) (vs. control; PBS) increased thromboxane A_2 analog-induced contraction, whereas decreased noradrenaline-induced contraction. The decreased noradrenaline-induced contraction by AGE-BSA was prevented by co-treated with organic cation transporter-3 (OCT-3) inhibitor corticosterone. The protein expression of OCT-3 in endothelium-denuded carotid artery was similar between control and AGE-BSA groups. These results suggest that ligand specific alterations of contractile responses by AGE-BSA exposure were seen in carotid arteries, and that decreased noradrenaline-induced contraction by AGE-BSA may be partly due to increased OCT-3 activity rather than the expression.

Effects of advanced glycation endproducts on uridine diphosphate-induced contraction in rat carotid artery

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Advanced glycation end-products (AGEs) play a pivotal role in vascular function in various (patho)physiological conditions. Although uridine diphosphate (UDP) is an important extracellular nucleotide, the direct relationship between AGEs and UDP regarding their effects on vascular functions remain unclear. Therefore, we investigated the effects of AGE-bovine serum albumin (AGE-BSA) on UDP-mediated responses in rat carotid arterial rings. Concentration–dependent contraction but not relaxation was obtained following UDP application to carotid arteries with and without endothelia; the contraction was greater in the AGE-BSA-treated (0.1 mg/mL for 60 min) group than the control (1.0 v/v% PBS) group. The difference in UDP-induced contraction between the control and AGE-BSA-treated groups was not abolished by L-NNA [a nitric oxide synthase (NOS) inhibitor], whereas the difference was abolished by indomethacin [a cyclooxygenase (COX) inhibitor], ozagrel [a thromboxane synthase (TXS) inhibitor], and by SQ29548 [a thromboxane-prostanoid (TP) receptor antagonist]. The release of TXB_2, a metabolite of TXA_2, was increased by UDP in both groups, whereas the levels were similar between two groups. The release of PGE_2, other vasoconstrictor prostanoid, was similar among the groups (UDP-stimulated or -unstimulated control/AGE-BSA-treated groups). The contraction induced by U46619, a TP receptor agonist, in the presence of L-NNA was increased in the AGE-BSA-treated group compared with the control group. We conclude that the increase in UDP-induced contraction in the presence of AGE-BSA can be attributed to an increase in the COX/TXS/TP receptor pathway, in particular, TP receptor signaling.

SIRT1, an NAD$^+$-dependent protein deacetylase, maintains oxidative muscle fiber in the skeletal muscle and contributes to exercise capacity in mice.

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[Background and Aim]

We previously reported that resveratrol, an activator of an NAD$^+$-dependent deacetylase SIRT1, improves exercise tolerance with upregulation oxidative type of muscle fibers in a mouse model of Duchenne muscular dystrophy. SIRT1 deacetylates and activates PGC-1α, a co-activator to promote expression of oxidative muscle fibers. Here, we examined whether SIRT1 maintains oxidative muscle fibers for exercise capacity in the skeletal muscle.

[Methods and Results]

We first compared the expression level of SIRT1 in several types of skeletal muscles in wild-type mouse (WT). The SIRT1 protein level was the highest in the soleus muscle (+3.5-fold), which is mainly made up of oxidative fibers, compared to the other glycolytic muscles such as quadriceps, gastrocnemius, tibial anterior, and extensor digitorum longus muscles. The SIRT1 mRNA level was most abundant also in the soleus muscle. Immunohistological analysis using soleus muscle sections showed that the percentage of type IIa, one of oxidative muscle fibers, was significantly lower in the skeletal muscle-specific SIRT1 knockout mouse (SIRT1MKO) than that in WT (42% vs. 56%) at 79-98 weeks of age. In contrast, the percentage of glycolytic type IIx+IIb fibers was higher in the SIRT1MKO (15%) compared to WT (11%). Treadmill running distance at 15 weeks of age was significantly shorter in SIRT1MKO (158 ±10 m) than that in WT (1088±33 m).

[Conclusion]

These results suggest that SIRT1 maintains exercise capacity by preserving oxidative muscle fibers in the skeletal muscle.

Liver blood flow is regulated by hepatic stellate cells and sympathetic nerve in the sinusoid

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The diameter of sinusoid has been shown to be changed by perfusion of adrenaline and acetylcholine. Thus, the liver blood flow is suggested to be regulated by the autonomic nervous systems in the sinusoid. However, it remains to be elucidated which cells in the sinusoid are involved in the response. The present study focused on hepatic stellate cells (HSCs) encircling the sinusoid and aimed to determine 1) whether HSCs constrict in response to noradrenaline (NAd) and 2) whether HSCs regulate liver blood flow. To measure HSC constriction quantitatively, we developed a novel method using fluorescent beads. We observed that HSCs constricted in response to NAd, which was suppressed by the α_1-adrenoceptor inhibitor bunazosin and the non-muscle myosin II inhibitor blebbistatin (Bleb;1 μM). In contrast, Bleb (1 μM) had no effect on the contraction of isolated portal veins. The NAd-induced constriction of HSCs was also suppressed by xestospongin C, YM-58483, W-7, ML-9, and H-1152. In addition, Bleb (1 μM) decreased the perfusion pressure of the liver increased by NAd. This response appears to be due to HSC relaxation, since Bleb had the inhibitory effect on HSCs but not on portal veins. These results suggest that NAd induces constriction of HSCs via increasing Ca^{2+} influx and Ca^{2+} sensitization, thereby regulating liver blood flow.

The roles of progesterone receptor membrane component 1 (PGRMC1) in cyclic AMP-induced human endometrial and trophoblast cells differentiation

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Differentiation of human endometrial stromal cells into decidual cells is indispensable for the embryo implantation during the mid-secretory phase of the menstrual cycle. After the implantation, placental villus cytotrophoblasts differentiate into syncytiotrophoblasts to form placenta. These differentiation events of ESCs and trophoblasts cells are induced by the activation of cyclic AMP (cAMP) signaling pathway. Progesterone (P4) receptor membrane component 1 (PGRMC1) is a member of a P4 binding complex implicated in female reproduction, however, its role in ESCs and trophoblast cells differentiation has not been examined. In this study, we explored the significance of PGRMC1 in cAMP-induced differentiation of ESCs and trophoblast cells. Treatment of ESCs with the cAMP analog dibutyryl (db)-cAMP repressed PGRMC1 expression. Both knockdown and inhibition of PGRMC1 significantly promoted the expression of differentiation markers such as IGF-binding protein 1 and prolactin in db-cAMP-stimulated ESCs. Furthermore, inhibition of PGRMC1 facilitated the production of human chorionic gonadotropin (HCG), which is the differentiation marker of syncytiotrophoblast in placental choriocarcinoma BeWo cells. These findings suggest the significance of P4-independent inhibitory action of PGRMC1 in cAMP-induced ESCs and trophoblast differentiation.

Hypoxia stimulates CXCR4, an EMT-related factor expression in endometrial epithelial cells

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Endometriosis is characterized by the ectopic inflammation, growth, and fibrotic changes in the lesion. Previous study indicates that peritoneal bleeding may accelerate inflammation partially through the activation of protease-activated receptor (PAR) and the prostaglandin (PG) EP2 receptor in endometriosis-like graft of the mouse model. To explore the involvement of hypoxia and/or the inflammatory mediators in epithelial-mesenchymal transition (EMT) of endometrial cells, we examined the effects of thrombin, a PAR agonist and PGE2 on EMT marker expression and cell migration in human endometrial stromal (EtsT499) and epithelial (EM-1) cells. The endometrial cells in 3D culture system incubated for 18 h under hypoxia were cultured in the presence or absence of the combined treatment of thrombin and PGE2 (Throm/PG) for 72 h. Hypoxic conditions increased expression of CXCR4, an EMT marker in EM-1, but not in EtsT499, whereas Throm/PG did not affect CXCR4 in both cells. Throm/PG treatment promoted the migration of EM-1 under hypoxia. Thus, Throm/PG stimulation under hypoxia enhanced CXCR4 expression and accelerated migration of the endometrial epithelial cells. Our data suggests that the inflammatory mediators in retrograde menstrual fluid may be associated with the pathophysiology of ectopic endometrial EMT and migration.

Comparative analysis of anti-diabetic effects of citrus flavonoids on pancreatic β-cell function

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The chronic hyperglycemia that occurs in type 2 diabetes causes deterioration of pancreatic β-cell dysfunction which involves a decrease in insulin secretory response and a decrease in β-cell mass. Thus, to promote β-cell function and survival would provide therapeutic approaches to prevent the onset and development of type 2 diabetes. Citrus flavonoids are known to have health benefits, especially those related to improvement of type 2 diabetes. However, little is known about the effects of these flavonoids on pancreatic β-cell functions. We have previously demonstrated that nobiletin has anti-diabetic effects on β-cell functions. Tangeretin and sudachitin are polymethoxy flavonoids (PMF) contained in citrus peel and have a similar structure to nobiletin. In the present study, we investigated the effects of the PMFs on glucose-induced insulin secretion (GSIS) and β-cell apoptosis in the β-cell line INS-1 and compared these effects with those of nobiletin. Tangeretin significantly increased GSIS at 10 μM and inhibited thapsigargin-induced apoptosis. Sudachitin also significantly increased GSIS at 100 μM but did not affect β-cell apoptosis. The anti-diabetic effects of tangeretin on β-cell functions were more potent than those of sudachitin, but they were less potent than those of nobiletin. These results suggest that nobiletin has more remarkable anti-diabetic effects on β-cells, i.e., more potent insulinotropic and anti-apoptotic effects, than tangeretin and sudachitin.

Attenuation of 5α-reductase-mediated progesterone metabolism promotes differentiation of human endometrial stromal cells for the establishment of pregnancy

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Human endometrial stromal cells (ESCs) differentiate into decidual cells during the mid-secretory phase of the menstrual cycle following the postovulatory rise in progesterone (P4). Progesterone (P4) is a predominant inducer of the differentiation which is essential for the establishment of pregnancy. In this study, we explored the roles of 5α-reductases-mediated P4 metabolism in the differentiation of ESCs induced by P4 and dibutyryl cAMP (P4/db-cAMP) treatment. The ability of P4 metabolism in differentiated ESCs was compared with that in undifferentiated cells. The residual P4 level in media was much higher in the differentiated ESCs than in control cells, whereas the amount of the P4 metabolite allopregnanolone was less in the differentiated cells. Treatment of ESCs and endometrial epithelial cells with the 5α-reductase inhibitors dutasteride and finasteride repressed P4 metabolism. Furthermore, inhibition of 5α-reductase facilitated expression of differentiation markers, IGF-binding protein 1 and prolactin in P4/db-cAMP-stimulated ESCs. The expression of *SRD5A1*, which encodes 5α-reductases type 1, was reduced in differentiated ESCs and epithelial cells. These data suggests endometrial 5α-reductase metabolizes P4 and the enzyme-mediated metabolizing pathway maybe involved in the increase in P4 level for promoting ESC differentiation.

Stimulatory effect of the extract of fruit body of *Cordyceps militaris* on the secretion of testosterone in rats

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Testosterone, primarily produced in Leydig cells, is essential for a variety of systemic functions, and deficiency of this hormone results in late-onset hypogonadism (LOH) in climacteric male. In the present study, we prepared the extract of fruit body of *Cordyceps militaris* parasitized in *Samia Cynthia ricini*, designated as Ryukyu-Kasou (RK), as a novel candidate for ameliorating male menopause symptom. To explore the effect of RK on LOH, we have investigated the testosterone dynamics using castrated rats and isolated primary rat Leydig cells. Testosterone propionate (TP) and the extract of RK were administered to castrated rats for 12 days. The serum levels of testosterone and dihydrotestosterone (DHT) were maintained highly in the RK-treated group, compared with control. In addition, RK reduced TP-stimulated increases in the weight of prostate gland. When cultured testicular cells were stimulated with luteinizing hormone (LH) or dibutyryl-cyclic AMP (cAMP) in the presence or absence of RK, LH- or cAMP-induced testosterone secretion in the media was enhanced by the presence of RK with no changes in the mRNA expression of steroidogenic enzymes. Thus, RK-derived unknown compounds suppressed the testosterone catabolism *in vivo* and stimulated testosterone secretion *in vitro*, suggesting the possibility of RK for improving LOH.

Establishment of high HPRT activity *Urat1-Uox* double knockout mouse and the effects of xanthine oxidoreductase inhibitor

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It is known that there are species differences in the purine metabolic system between humans and rodents (e.g. urate oxidase (Uox), and hypoxanthine phosphoribosyltransferase (HPRT), etc.). URAT1 (SLC22A12) is renal urate (UA) reabsorption transporter and the target for UA-lowering therapies. In humans, URAT1 deficiency has a significant UA-lowering effect (ULE), but not in *Urat1*-knokout (KO) mice. The aim of this study is the establishment and urate kinetic profiling of high HPRT activity *Urat1-Uox* double knockout (DKO) mice and the investigation of the effect of xanthine oxidoreductase inhibitor (XOI), topiroxostat in this model mice. Topiroxostat 1 mg/kg (Top) was administered to DKO mice for 7 days by feeding diet. Oxypurines (UA, hypoxanthine and xanthine) and creatinine in plasma and urine were measured by HPLC. DKO mice showed a significant decrease in plasma UA levels, increased fractional excretion of UA (FE_{UA}), and enhanced Top-induced ULEs, compared with *Uox*-KO only. Thus, high HPRT activity seems to be important for producing ULE by URAT1 inhibition. The combination therapy of URAT1 inhibition and XOI showed an effective ULEs, suggesting that it is useful for the treatment of hyperuricemia.

Preparation of peripheral blood-derived microglia-like cells and its intra-hippocampal injection to ameliorate amyloid-β burden and cognitive impairment in a mouse model of Alzheimer's disease

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Amyloid-β (Aβ) accumulation in the brain is the first trigger for the onset of Alzheimer's disease (AD), and its prevention and elimination are promising strategies for AD therapy. Previously, we demonstrated that injection of mouse bone marrow (BM)–derived microglia-like (BMDML) cells into the brain decreases Aβ and ameliorates cognitive impairment in a mouse model of AD. In this study, considering majority of AD patients are elderly and less invasive ways for preparing autologous microglia-like cells are needed, we focused on hematopoietic stem cells (HSCs) in peripheral blood (PB). Mouse HSCs were mobilized from BM to PB by administration of granulocyte colony-stimulating factor (G-CSF) and CXCR4 antagonist and were collected from PB. Collected HSCs were subsequently differentiated into microglia-like cells upon stimulation with colony- stimulating factor 1 (CSF-1) and interleukin-34. The PB-derived microglia-like (PBDML) cells expressed macrophage/microglia markers and effectively phagocytosed Aβ. We further found that PBDML cells injected into the hippocampi of AD model mice diffused in the brain with phagocytosing Aβ, and contributed to the reduction of brain Aβ and improvement of cognitive impairment. These results suggest that PBDML cells could be promising candidate source for the development of cell therapy against AD.

Loss of $P2Y_1$ receptor causes ocular hypertension and glaucoma-like phenotype in mice

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Glaucoma is second leading cause of blindness worldwide which is characterized by progressive degeneration of retinal ganglion cells (RGCs). Elevated intraocular pressure (IOP) is one of the highest risk factors and IOP-lowering agents are used to prevent glaucoma. New molecular target is required because of the side effects, drug resistance, and insufficiency for IOP reduction by a part of pre-existing agents. Here, we report that $P2Y_1$ receptor ($P2Y_1R$) activation induces IOP reduction and knock out of $P2Y_1R$ ($P2Y_1KO$) causes sustained IOP elevation associated with age-dependent RGC degeneration. Topical application of MRS2365, selective agonist for $P2Y_1R$, caused significant reduction in IOP in wild-type (WT) mice but not in $P2Y_1KO$ mice. We also found that $P2Y_1KO$ mice showed significantly higher IOP level than that in WT mice. Because sustained IOP elevation is one feature of hypertensive glaucoma, we checked RCG damages and found that the number of RGCs in $P2Y_1KO$ mice was comparable at 3 months old but significantly smaller at 12 months old. Furthermore, optical coherence tomography (OCT) revealed that 12-month-old $P2Y_1KO$ mice showed thinner ganglion cell and inner plexiform layers, general diagnostic feature of glaucoma patients. Taken together, our results demonstrated that (1) $P2Y_1R$ activation reduces IOP; (2) loss-of-function of $P2Y_1R$ causes sustained elevation in IOP and (3) hypertensive glaucoma-like phenotypes in middle-aged mice.

1-SS-19 Student Sessions

The role of a novel hyaluronan depolymerization factor, HYBID, on glioma

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HYBID (hyaluronan binding protein involved in hyaluronan depolymerization) is a novel factor associated with hyaluronan depolymerization. HYBID facilitates the several tumor progression and the expression level of HYBID is helpful as a predictor of tumor progression including colon and pancreatic tumor. Though HYBID is important for hyaluronan metabolism in brain, there is no report on glioma. Therefore, we evaluated the role of HYBID and hyaluronan on glioma using *in vitro* and *in vivo* glioma models.

First, we evaluated the cell proliferation, migration, and the expression of some related proteins after knock of *hybid* by using siRNA in U251 human glioma cell. Moreover, we evaluated the tumor size by using the *in vivo* glioma model with HYBID KO and WT mice. Murine glioma model was estimated by hematoxylin and eosin staining.

Hybid knock down suppressed the glioma cell proliferation, migration and Wnt/β-catenin signal related protein. HYBID may promote the glioma progression *via* Wnt/β-catenin signal. Moreover, tumor size in HYBID KO mice were smaller than that in HYBID WT mice. This result indicates that host derived HYBID is contributed to glioma progression.

In conclusion, these findings indicate that HYBID was an important factor for glioma progression.

Stimulation of LXA_4 receptor alleviates motor dysfunction in intracerebral hemorrhage model mice

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Intracerebral hemorrhage (ICH), a bleeding into the brain parenchyma, is a devastating neurologic disease with the highest mortality among all stroke subtypes. In ICH brain, thrombin induces activation of microglia/macrophages followed by neuroinflammation. Furthermore, ICH leads to infiltration of numerous leukocytes. Recent report shows the arachidonic acid metabolite, leukotriene B_4 (LTB_4), participates pathological progression of ICH (Hijioka *et al.*, 2017). In this study, we focused on lipoxin A_4 (LXA_4), synthesized from arachidonic acid as same as LTB_4. Treatment of murine microglial cell line BV-2 cells with thrombin (30 U/mL) increased mRNA expression level of inducible NO synthase (iNOS) and interleukin-6 (IL-6). Pretreatment with LXA_4 (100 μM) suppressed thrombin-induced increases in iNOS and IL-6 mRNA expression. Moreover, immunocytochemical analysis revealed the translocation of nuclear factor-κB (NF-κB) into the nucleus induced by thrombin, and thrombin-induced nuclear translocation of NF-κB was suppressed by LXA_4. Finally, daily intravenous administration of LXA_4 receptor agonist, BML-111 (1 mg/kg) attenuated the motor dysfunction of mouse model of ICH. These data suggest that LXA_4 may be the novel therapeutic agent for ICH.

The effect of anthocyanin-rich blackcurrant extracts on behavioral abnormalities in SAMP8 mice

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Anthocyanins possess high antioxidant activity and are the major group of polyphenols in blackcurrant, a regional specialty in Aomori prefecture. In this study, we investigated the effect of black currant extracts on cognitive and emotional abnormalities in the senescence-accelerated mouse prone 8 (SAMP8). Four month-old SAMP8 mice were fed a basal diet supplemented with 3% blackcurrant extracts for 2 months, and then behavioral experiments were conducted. In the novel object recognition test, treatment with blackcurrant extracts improved the memory impairment in SAMP8 mice. In addition, reduced anxiety-like behavior in SAMP8 mice was reversed by the extracts in the elevated plus maze test. These results suggest that supplementation of blackcurrant extracts has the potential to improve cognitive and emotional abnormalities in aging as well as age-related neurodegenerative diseases such as Alzheimer's disease.

Establishment of the method of three-dimensional organoid culture derived from liver tissues of non-alcoholic steatohepatitis (NASH) model mice

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【Background】

Nowadays, there are many non-alcoholic steatohepatitis (NASH) patients who develop fatty liver without alcohol. NASH patients usually progress to liver cirrhosis or liver cancer in the future. However, the detailed pathogenic mechanisms still have not been clarified. Therefore, a new approach is required to establish the therapeutic method of NASH disease.

【Object】

To clarify the usefulness of organoids derived from liver tissue of NASH model mice, we examined the correlation between the histopathology of disease progression of NASH mice and the function and histology of liver organoids.

【Methods】

Seven-week-old C57BL / 6J mice were fed a diet for 4, 8, and 12 weeks to induce different stages of NASH disease. After measuring liver weight and blood parameters of NASH mice, the histopathological structure of liver tissue was analyzed. Using isolated the liver tissues, we generated liver organoids and checked the histopathology and functions of them.

【Result and discussion】

Liver organoid formation and expression of hepatocyte markers, albumin, AFP and CYP3A4 / 5 were observed in all groups of NASH mice. Liver organoids from mice fed a NASH diet for 4 weeks showed the highest organoid-forming ability. And liver organoids from mice fed a NASH diet for 12 weeks showed epithelial-mesenchymal transition with a decrease in intercellular adhesion and an increase in collagen I expression. These results suggest that liver organoids derived from NASH model mice may recapitulate the characteristics of liver fibrosis and become a new research tool for NASH disease.

Thromboxane A2 receptor signaling attenuates monocrotaline-induced liver injury by affecting endothelial cells, but not platelets

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Sinusoidal obstruction syndrome (SOS) is a major complication of chemotherapy and hematopoietic stem cell transplantation. The early stage of SOS is characterized by liver sinusoidal endothelial cell (LSEC) injury accompanied by platelet aggregation. Thromboxane A_2 (TxA_2) induces platelet aggregation through the thromboxane prostanoid (TP) receptor. In this study, we explored the role of TP signaling in a monocrotaline (MCT)-induced model of SOS using male C57BL/6 mice (wild type) and TP-deficient ($TP^{-/-}$) mice. Relative to WT mice, $TP^{-/-}$ mice exhibited more severe MCT-liver injury, as indicated by elevated levels of alanine aminotransferase (ALT) and coagulative necrosis. Extensive accumulation of platelets in the liver was observed in both WT and $TP^{-/-}$ mice; however, there was no significant difference between the genotypes. TP expression co-localized with CD31-positive LSECs. MCT treatment caused LSEC destruction, concomitant with elevated expression of matrix metalloproteinases (MMPs) and adhesion molecules in WT mice, and LSEC damage was further exacerbated in $TP^{-/-}$ mice. Viability of isolated LSECs stimulated with MCT from $TP^{-/-}$ mice was lower, whereas mRNA levels of MMPs and adhesion molecules were higher; U46619, a TxA_2 agonist, reduced these levels in WT mice. These data suggest that TP signaling has no effect on platelet accumulation during MCT-induced liver injury, but instead prevents injury by suppressing LSEC damage.

DAIKENCHUTO, A TRADITIONAL JAPANEASE (KAMPO) MEDICINE, FACILITATES CONTRACTION IN ISOLATED MOUSE DISTAL COLONS: INVOLVEMENT OF TRPA1 CHANNEL AND CALCITONIN GENE-RELATED PEPTIDE

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AIM: Daikenchuto (DKT) improves symptoms associated with postoperative ileus and constipation. we have Previously shown that allyl-isothiocyanate, a TRPA1 activator, induces contraction in mouse distal colon via primary sensory nerves and cholinergic neurons. We investigated the effect of DKT on colonic motility in the isolated mouse distal colon. Especially, we focused on the role of TRPA1 channels and CGRP receptors. **METHODS:** Distal colons were isolated from male ddY mice. The longitudinal smooth muscle tension was isotonically measured by using Magnus apparatus. **RESULT:** DKT induced twitch contraction in dose-dependent manner (0.1-10 mg/mL). The maximal response was observed at 3 mg/mL. DKT-induced contraction was inhibited by the NK_1 antagonist FK888, the NK_2 antagonist GR159897, and the CGRP receptor antagonist BIBN4096. The TRPA1 blocker A9607079 also markedly inhibited the contraction. The contraction was abolished by the pretreatment with atropine or tetrodotoxin. In the immunohistochemical study, TRPA1 immunoactivities were found in muscle layers of the mouse distal colon. Several TRPA1-expressing nerve fibers contained substance P (SP) and CGRP. **CONCLSION:** The DKT induced twitch contraction was mediated by TRPA1-expressing sensory nerves, which release SP and CGRP from the nerve terminals.

Establishment of novel post-inflammatory irritable bowel syndrome model mice with dextran sulfate sodium

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AIM : The purpose of this study is to develop the post-inflammatory irritable bowel syndrome (PI-IBS) animal model after a healing of dextran sulfate sodium (DSS)-induced inflammatory bowel disease. **METHODS :** C57BL / 6J male mice were used. PI-IBS model was prepared by drinking freely 3% DSS until day 4, and then changing drinking water to tap water during recovery period. The myeloperoxidase (MPO) activity in the colon was measured to determine the levels of inflammation. Histological analysis was done by using HE staining. **RESULTS :** The severe tissue damage in colonic mucosa was observed on day 4, but these damage disappeared on day 14 and colonic mucosa recovered to normal. Although MPO activity on day 4 in PI-IBS model group showed a significant increase as compared to in the normal group, the activity on day 14 reduced to the normal levels. In pain experiment, an increase in visceral pain-like behaviors in PI-IBS model mice was observed on day 4, and the pain-like behaviors observed until day 14. **CONCLUSIONS :** We have established novel PI-IBS model mice that possess persistent visceral hyperalgesia without of no tissue damage.

Involvement of thermo-sensitive TRPM8 channels in visceral hypersensitivity in irritable bowel syndrome animal models

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AIM: We prepared two models, namely butyrate-induced irritable bowel syndrome (IBS) model rats and a post-inflammatory IBS (PI-IBS) model mice using dextran sulfate sodium (DSS). In the present study, we investigated the involvement of TRPM8 in visceral hyperalgesia in the two IBS models. **METHODS:** Butyrate-induced IBS model was prepared by intracolonic treatment with butyrate for 4 days in SD male rats. PI-IBS model was prepared by drinking freely 3% DSS until Day 4, then changing 3% DSS to tap water during recovery period. Visceral pain was induced by intracolonic treatment with the TRPM8-selective agonist WS-12, and the observed visceral pain-like behaviors ware mesured. TRPM8 immunoreactivities were detected using immunohistochemical techniques. **RESULTS:** In rectal histology of butyrate-induced IBS model rats, the number of TRPM8-expressing nerve fibers in the mucosal layer ware significantly increased compared with normal rats. In behavioral observation, WS-12-induced visceral pain-like behaviors were increased. In PI-IBS model mice, an increase in visceral pain-like behaviors was observed on Day 4, and the pain symptom observed until Day 14. **CONCLUSIONS:** These results suggest that the increase of TRPM8-expressing nerve fibers in lower gastrointestinal tracts is involved in hyperalgesia in IBS model animals.

Drug-induced arrhythmia prediction method based on voltage-dependent I_{CaL}block

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Drug-induced arrhythmia can occur under prolonged action potential duration (APD) due to block of I_{Kr}. Therefore, I_{Kr} block and APD prolongation have been used for predicting drug induced arrhythmia.However, I_{Kr} blockers have difference in risk for drug-induced arrhythmia. One of the reasons is that the occurrence of drug-induced arrhythmia under bradycardia is initiated by early afterdepolarization (EAD) at the repolarization phase in prolonged action potential. For example, terfenadine, which prolongs APD and cause EAD, is considered as a drug with a high risk for drug-induced arrhythmia. On the other hand, amiodarone, which prolongs APD but does not cause EAD in clinical practice, has been considered as a relatively safe antiarrhythmic drug. Therefore, there is a possibility that EAD occurrence can account for the difference in the risks among I_{Kr} blockers. To study the mechanisms underlying different occurrence of EAD, we examined the effects of voltage-dependent I_{CaL} block property on EAD. In the present study, we used a mathematical model of human ventricular action potential. The results showed that amiodarone-like I_{CaL} block model suppressed EAD. But, I_{CaL} block models of terfenadine-like and bepridil-like increased EAD occurrence. The different effects on EAD were accounted for by difference in voltage-dependent block of I_{CaL}, as weak I_{CaL} block in hyperpolarized potential increased the occurrence of EAD. Therefore, to predict drug-induced arrhythmia, not only APD prolongation but also voltage-dependent property of I_{CaL} block should be checked.

Acute intracerebroventricular injection of chemerin-9 increases systemic blood pressure via CMKLR1 in rats

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Chemerin is an inflammatory adipocytokine through acting on G protein-coupled receptor, chemokine-like receptor (CMKLR)1. We previously demonstrated that long-term intraperitoneal administration with chemerin increased systolic blood pressure (BP) in mice. Since important nuclei regulating BP exist in the brain, we examined mechanisms of central BP control by chemerin-9.

BP was invasively measured after acute intracerebroventricular (i.c.v.) injection of chemerin-9 to CMKLR1 siRNA-treated rats for 3 days. Serum adrenaline was measured by HPLC method. CMKLR1 expression in the brain tissues of spontaneously hypertensive rat (SHR) was examined by Western blotting.

In the control siRNA-treated rats, chemerin-9 significantly increased mean BP, while it had no effect in the CMKLR1 siRNA-treated rats. Serum adrenaline level was increased by the acute i.c.v. injection of chemerin-9. CMKLR1 expression around brain ventricles from SHR was increased.

We for the first time demonstrated that chemerin-9 stimulates the sympathetic nerves via CMKLR1 expressed around brain ventricles, which leads to an increase in BP. It is further suggested that chemerin/CMKLR1 in the central nerves plays an important role in essential hypertension.

Comparison of transplantation effects of cardiac progenitor cell types on the mitochondrial energy-producing ability after myocardial infarction in rats

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The cardiosphere-derived cell (CDC) is one of the candidate cells used for cardiac regenerative therapy. Cardiospheres are mixture of CDCs including c-Kit$^+$ cells, Sca-1$^+$ cells, and other types of cardiac progenitor cells. In this study, we compared effects of transplantation of isolated Sca-1$^+$ cells and c-Kit$^+$ cells with that of the crude CDCs (CrCDCs). We found that the transplantation of these 3 types of cells resulted in a preservation of the cardiac pump function and mitochondrial respiration. However, mitochondrial function in the c-Kit$^+$ cell-transplanted group was lower than that in the other 2 experimental groups. Furthermore, we found that activation levels of intracellular signaling proteins after cell transplantation may have been development on the ability of secretion of several growth factors, such as IGF-1 and TGF-b, by these transplanted cell types. Our findings suggest the possibility that CrCDC and Sca-1$^+$ cells rather than c-Kit$^+$ cells are preferable for the therapy to preserve cardiac function and energy metabolism after myocardial infarction.

Effects of quercetin against inflammation and endothelial dysfunction in the aortas from aneurysm model mice.

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Loading of angiotensin II (Ang II) and β-aminopropionitrile (BAPN) to mice is known to cause hypertension and degeneration of elastic lamina, resulting to the onset of aortic aneurysm (AA). As one of the pathogenesis, inflammation at the aortic wall and endothelial cell injury are observed in these mice. Quercetin, which is abundant in onion, is reported to improve vascular function. In this study, we investigated the effects of quercetin on vascular injury such as inflammation and endothelial dysfunction. In C57BL/6J male mice, Ang II and BAPN were administered via osmotic mini pumps. Quercetin was administered from 2 weeks prior to the start of Ang II and BAPN loading. Isolated aorta was subjected to the analysis protein and mRNA expressions. Quercetin treatment reduced the expressions of VCAM-1 and F4/80, a marker of macrophages in aortas as well as the incidence of AA in mice. For *in vitro* study, cultured human umbilical vein endothelial cells (HUVECs) were used. Quercetin also inhibited VCAM-1 expression increased by TNF-alpha in HUVECs. Moreover, quercetin activated ERK5, which is known as an endothelial cell protective molecule in HUVECs. In the present study, quercetin shows anti-inflammatory and endothelial cell protective effects in both aortas and HUVECs and may prevent the AA onset.

Roles of Hsp90 on cardiac remodeling in the development of chronic heart failure.

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Hsp90 is a highly conserved molecular chaperone involved stabilization of client proteins. Hsp90 clients are various signal transducers including protein kinases. It is well known that the cardiac remodeling such as cardiac hypertrophy and fibrosis is involved in the development of chronic heart failure. The cardiac remodeling is regulated by many signaling pathways. c-Raf and JNK are signal transducers involved in cardiac remodeling, and are also Hsp90 client proteins. Therefore, to clarify the roles of Hsp90 and its clients in progression to the chronic heart failure, we examined effects of Hsp90 inhibitor on the signal transducers in the development of chronic heart failure in animal models. Treatment of the animals with Hsp90 inhibitor resulted in a suppression of cardiac remodeling and a preservation of cardiac pump function. Furthermore, c-Raf/Erk signaling was attenuated by an administration of Hsp90 inhibitor. These results suggest that Hsp90 contributes to stabilization of several cardiac remodeling-associated protein kinases during the development of chronic heart failure.

Relationship between brain oxidative stress and autistic behaviors in autism spectrum disorder rats

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[Background] Autism spectrum disorders (ASD) belong to neurodevelopmental diseases characterized by social deficits, repetitive behaviors, and learning disability. Although ASD are pathogenetically heterogeneous and complex as the causes are diverse genetically and environmentally, oxidative stress and mitochondrial dysfunction are associated with ASD brain pathology. We here addressed whether oxidative stress and mitochondria in the brain could be targets for ASD therapeutics, using prenatal valproic acid (VPA)-exposed rats. **[Methods]** After prenatal exposure to VPA (600 mg/kg, p.o.) on embryonic day 12.5, rats were subjected to behavioral tasks to assess social behaviors, learning and memory at adolescence. Then the dorsal hippocampus was collected and oxidative damage and mitochondrial function were measured. **[Results]** Social behaviors, spatial reference memory, and object recognition were impaired in VPA-exposed rats like ASD patients. Immunohistochemical analyses using 4-hydroxy-2-nonenal antibody revealed that oxidative stress is increased in the dorsal hippocampus of VPA-exposed rats, and this was accompanied by aberrant enzymatic activities of mitochondrial transport chain and reduced ATP levels. Chronic treatment of intranasal oxytocin (12 μg/kg) improved these ASD-like behaviors but not oxidative stress or mitochondrial dysfunction. In contrast to oxytocin, oral 5-aminolevulinic acid (5-ALA; 30 mg/kg), a mitochondrial heme precursor, attenuated not only ASD-like behaviors but also oxidative stress and mitochondrial dysfunction seen in VPA-treated rats. **[Conclusion]** Oral 5-ALA treatment improves ASD-like behaviors like intranasal oxytocin administration. These results indicate that 5-ALA could ameliorate ASD symptoms through amelioration of oxidative stress and mitochondrial dysfunction, of which mechanisms are different from oxytocin.

The cognitive function of the mice with an exonic deletion of RELN is impaired in touchscreen-based visual discrimination task

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Touchscreen-based cognitive tasks have been developed for rodents to provide a better translational approach across species for further understanding of the cognitive impairment observed in various neuropsychiatric disorders. Reelin protein (RELN), an extracellular matrix protein, plays an important role in embryonic neuronal migration and the development of the laminar structure of the cerebral cortex. In the present study, we aimed to explore the performance of RELN-deletion (include exons 52 to 58) mice, using a touchscreen-based visual discrimination (VD) task. Mice were initially trained to discriminate between a pair of stimuli simultaneously displayed on the screen and received a liquid reward. The cognitive function was impaired in RELN deletion mice as demonstrated by the increased total, total errors, total correction trials and total sessions in complex VD task and complex reversal learning. We demonstrated that cognitive function is impaired in RELN-deletion mice.

Effect of new preventive medicine on pentylenetetrazol-induced kindled mice using database analyses

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Objective: Despite the availability of more than 20 antiepileptic drugs in clinical, approximately 30 percent of people with epilepsy do not respond to antiepileptic drugs treatment. It is important to develop antiepileptic products with new mechanisms. At present, we also evaluated data from one of the largest global database to find drugs with antiepileptic effects. Therefore, the present study was undertaken to clarify the effect of combination of anti-epileptic drugs and valacyclovir in epileptic seizure using kindling model.

Methods: To induce kindling, pentylenetetrazol at a dose of 40 mg/kg was injected once every 48 h. Behavioral seizures were monitored for 20 min following pentylenetetrazol administration. In this study, valacyclovir was orally administered 30 min before antiepileptic drugs injected in kindled mice.

RESULTS: Valacyclovir showed inhibitory effects on pentylenetetrazol -induced kindled seizures. In addition, simultaneous use of levetiracetam and valacyclovir caused more potent inhibition of seizure activities. The other hand, the inhibitory effect of valproic acid or diazepam with valacyclovir was not augment epileptic seizure in kindled mice.

CONCLUSIONS: The findings of the present study indicate that a combination of levetiracetam and valacyclovir show anticonvulsive effects on pentylenetetrazol -induced kindled epileptic seizures. These results suggested that valacyclovir may have antiepileptic effect in patients with epilepsy.

Alzheimer's drug candidate, SAK3 enhances the proteasome activity and inhibits amyloid aggregation in Alzheimer‘s disease brain

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[Background and Objectives] Alzheimer's disease (AD) is a progressive neurodegenerative and the most common disease of elderly dementia in the world. Acetylcholinesterase inhibitors such as donepezil and rivastigmine are the most useful drug for AD, but they are only used as symptomatic treatment and not disease-modifying drugs. We here developed a disease-modifying drug, SAK3 which stimulates T-type calcium channels and ameliorates AD pathology. Here, we introduce the novel mechanism through proteasome activation to inhibit Aβ deposition in *App*$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ knock-in (NL-G-F) mice.

[Methods] NL-G-F mice were chronically administered with SAK3 (0.5 mg/kg, p.o.) for 3 months. (i) Proteasome activity was assayed by fluorogenic peptides. (ii) Immuno-blotting analyses were conducted to assess CaMKII-Rpt6 pathway. (iii) The number and morphology of spine were assessed *in vivo*. (iv) Behavioral analyses were carried out to assess cognitive functions.

[Results] (i) The decreased 26S proteasome activities in the cortex of NL-G-F mice were restored by SAK3 chronic administration. (ii) The declined levels of phospho-CaMKII and phospho-Rpt6 in the hippocampus of NL-G-F mice were restored by SAK3 administration. (iii) The decreased number and abnormal morphology of spine in the cortex and hippocampus of NL-G-F mice were improved by SAK3 administration. (iv) The impaired cognitive function in NL-G-F mice was improved by SAK3 treatment.

[Conclusions] We defined the novel mechanism of SAK3-induced proteasome activation, thereby inhibiting the amyloid aggregation in AD mice. These results strongly suggest that SAK3 is the disease-modifying therapeutics for AD.

Neuroinflammation aggravates spreading alpha-synuclein oligomerization in Lewy body dementia mice

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[Background and Objectives] Accumulation and aggregation of alpha-synuclein in mid brain and cortex are causative for neuronal death in Lewy body with dementia (LBD). We found that the pathology is partly regulated by long-chain polyunsaturated fatty acids (LCPUFAs) such as arachidonic acid (AA) (1, 2) and brain inflammation. For example, fatty acid binding protein 3 (FABP3, H-FABP) is critical for AA-induced alpha-synuclein oligomerization (1, 2). However, the pathophysiological relevance of FABP3 and neuroinflammation remains unclear in alpha-synuclein spreading mechanism. We here documented the effect of neuroinflammation in the alpha-synuclein fibril-injected LBD mice. **[Methods]** To address the effects of neuroinflammation in alpha-synuclein spreading, we developed alpha-synuclein spreading model mice with or without LPS administration. The alpha-synuclein fibrils are injected into the dorsolateral striatum and its spreading was assessed by immunohistochemistry. **[Results]** At two months after alpha-synuclein fibril injection, mice exhibited cognitive impairment in novel object recognition task. The phosphorylated alpha-synuclein was detected in the cerebral cortex within two months. The single administration of lipopolysaccharide (LPS) before alpha-synuclein injection aggravated the phosphorylated alpha-synuclein accumulation. The neuroinflammation-induced morphological change in astrocytes was also evident in LPS-treated group. **[Conclusions]** The alpha-synuclein injection into striatum in mice is useful for screening LBD therapeutics which inhibit spreading and aggregation of alpha-synuclein. The LBD pathology is aggravated by neuroinflammation induced by LPS. This research is partially supported by AMED (19dm0107071)(http://lewybody2016.jp/). The authors declare no conflict of interests. (1) Shioda N et al., *J Bilo Chem* 2014;289:18957-18965. (2) Cheng A et al., *Brain Res*. 2019;1807:1980-197.

Facilitation of Schwann cell differentiation can be a novel approach to suppress paclitaxel-induced peripheral neuropathy

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We have previously demonstrated that paclitaxel reduces myelin-forming Schwann cells due to dedifferentiation of mature Schwann cells, prior to the induction of cytotoxicity against peripheral neurons. This cytotoxic process should be a causative pathogenesis of taxane-related chemotherapy-induced peripheral neuropathy (CIPN). To find the causal treatment of CIPN, we screened approved drugs with the ability to promote Schwann cell differentiation. Among numerous medicines,the most effective compounds identified was a PDE inhibitor, which promoted differentiation of immature Schwann cells, as indicated by increased expression of a mature Schwann cell marker, MBP. In a mixed culture of Schwann cells and DRG neurons, the co-treatment with a PDE inhibitor (30 mM) significantly suppressed paclitaxel (10
nM)-induced loss of myelin-forming Schwann cells (i.e. demyelination). In addition, the long-term administration of a PDE inhibitor (0.3% in chow diets) during the paclitaxel injection-period, paclitaxel (5 mg/kg, i.p.) twice a week for 8 weeks, reduced mechanical hypersensitivity in mice. Thus, we propose here that a PDE inhibitor, which prevents paclitaxel-induced Schwann cell dedifferentiation and demyelination, can be a therapeutic candidate to suppress paclitaxel-related CIPN.

1-SS-38 Student Sessions

Functional analysis of primary ciliogenesis related factors in zebrafish

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Primary cilia are nonmotile, 1–10 μm long antenna like structures observed in a variety of vertebrate cells. Primary cilia detect extracellular cues, such as mechanical flow and chemical stimulation, and transduce these signals into the cell. Therefore, the dysregulation of primary cilia can cause various diseases, including congenital anomalies, neurodevelopmental disorders, obesity, and cancer.

We have reported the regulation mechanism of primary cilia formation via the ubiquitin-proteasome system, and have identified a novel gene involved in primary cilia. These genes have been suggested to be associated with ciliopathy (a general term for diseases caused by primary ciliary abnormality), and the search for drugs that improve ciliopathy has been developed internationally. In this study, we will conduct a phenotypic analysis using zebrafish and examine what phenotypes are exhibited in zebrafish in which genes involved in the formation of primary cilia are knocked out. Phenotypic analysis in zebrafish is thought to be useful for exploring new treatments and drugs for ciliopathy.

Establishment of a drug screening system for chondrogenic differentiation of mesenchymal stem cells.

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Defects in articular cartilage ultimately results in loss of joint function in rheumatoid arthritis. To investigate the influences of anti-rheumatoid drugs on chondrogenic differentiation of mesenchymal stem cell (MSC), *in vitro* and *in vivo* screening system were established. MSCs were collected and palleted in 4-microtube strips for chondrogenic induction. Quantification of formed micromasses was performed by three dimensional T2-weighted magnetic resonance imaging. For *in vivo* screening, scaffold or scaffoldless cartilaginous tissue were transplanted to NOD/ShiJic-scid mice for 2 months. The cartilaginous tissues were then explanted and the expressions of chondrogenic markers, including aggrecan and CD44, were assessed. We examined influence on inducted differentiation cartilages by methotrexate (MTX) and prednisolone (PSL). The volume of the chondrogenic spheroid in the presence of MTX was decreased in a dose-dependent manner, whereas no significant effect of PSL on chondrogenic differential potency were observed. The MSC-derived cartilaginous spheroid provides an effective screening tool to get the impact of anti-rheumatoid drugs on cartilaginous regeneration.

Elucidation of neurite outgrowth by Wnt5a Ca^{2+}-dependently produced in periodontal ligament cells

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Sensory nerves in the periodontal ligament (PDL) sense the occlusal pressure, and contribute to adjust the occlusal force. The occlusal mechanical stimulation is necessary for nerve maturation in the PDL (Miki et al., 2015). Since there is no report on the regulatory mechanisms of peripheral nerve elongation in PDL, we focused on the neurotrophic factors and axon guidance proteins produced from the mechanically stimulated PDL cells.

The primary rat PDL cells were seeded on silicon chamber, and loaded with periodic mechanical stimulation (0.5 Hz, 15% expansion). The supernatant media of the mechanically stimulated rPDL cells elongated the neurite of the mouse primary TG and Me5 cells which are primary afferents from the PDL cells. The qPCR analysis of the mechanical stimulated PDL cells revealed that the expression level of *Wnt5a* was increased. The depletion of extracellular Ca^{2+} and the addition of Cd^{2+} suppressed the increase of *Wnt5a*. Besides, the PDL cells expressed the TRP C family, TRPV1, 2, 4 and Piezo1/2. Ruthenium Red inhibited the increase of *Wnt5a*. These results suggest that the mechanically stimulated PDL cells produce Wnt5a in a Ca^{2+} dependent manner, and the Wnt5a elongates the axon.

The activation of mouse hepatic stellate cells is suppressed by DIF-1, a morphogen produced by cellular slime molds.

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Hepatic stellate cells (HSCs), located in the gap of hepatocytes and sinusoidal endothelial cells, transdifferentiate from quiescent form (qHSCs) into myofibroblast-like activated one (aHSCs) during liver injury. The expression of α-smooth muscle actin (α-SMA) and the production of type Ⅰ collagen are up-regulated in aHSCs. Therefore, the activation of HSCs is responsible for liver fibrosis and inhibiting the activation can be a novel therapeutic target for the fibrosis. In the present study, we show that differentiation-inducing factor-1 (DIF-1) that is a low molecular weight compound derived from the cellular slime mold, *Dictyostelium discoideum*, has a suppressive effect on HSC activation. qHSCs were isolated from ddY mice and cultured in DMEM supplemented with 10% FBS. We treated qHSCs with DIF-1 on the next day after isolation and analyzed the effect of DIF-1 on HSC activation. DIF-1 significantly suppressed the up-regulation of α-SMA. However, the effect of DIF-1 was abolished in the presence of TWS119, an activator of Wnt/β-catenin signal pathway. DIF-1 reduced the levels of non-phosphorylated β-catenin (activated β-catenin) and phosphorylated GSK3β. These results suggest that DIF-1 inhibits the Wnt/β-catenin signal pathway through dephosphorylating GSK3β, thereby suppressing HSC activation.

1-SS-42 Student Sessions

Effects of estrogen on septic inflammatory responses in skeletal muscle

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Sepsis is a potentially fatal or life shortening syndrome due to infection induced systemic inflammatory responses. Numerous experimental and clinical studies indicate sex differences in sepsis. The outcome and survival rates from sepsis are better in women than in men. Morbidity due to sepsis is complicated by myopathy, and patients face long-term disability due to muscle atrophy and paralysis called intensive care unit acquired weakness (ICU-AW). Here, we examined the effects of estrogen on the septic inflammatory responses in skeletal muscle. 17β-estradiol (E2) attenuated muscle weakness induced by polymicrobial sepsis in ovariectomized mice. Furthermore, E2 attenuated atrophy, and inflammatory cytokine productions induced by lipopolysaccharide (LPS), an endotoxin in C2C12 myotubes. On the other hand, E2 did not change proteolysis pathways such as LPS induced atrogin-1/MAFbx up-regulation and autophagosome formation in C2C12 myotubes. These findings indicate that E2 protects skeletal muscle from septic damage by reducing inflammatory cytokines. According to this study, estrogen should be one of the factors of sex difference in sepsis. Compounds with estrogen-like action may be potential seeds for drugs for ICU-AW treatment.

The effects of Histidine-Rich Glycoprotein(HRG) on neutrophil-like differentiated cell lines

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Background: Our previous study revealed that HRG treatment ameliorated the survival rate of septic mice due to suppressing the excess immunothrombus formation. These results suggested that HRG may be one of the most useful drug for sepsis. However, it is difficult to obtain the stable experiment system for standardization of HRG drug product using neutrophils because of the short survival time and individual differences. In the present study, we examined whether the differentiated neutrophil-like cell lines showed the similar responses by HRG compared with human purified neutrophils. **Method:** All trans retinoic acid (ATRA) induces differentiation of the human myeloid leukemia cell lines HL-60 and NB-4. These cells were treated with Hank's Balanced Salt Solution (HBSS), human serum albumin (HSA), or HRG. The effects of HRG on these cells were evaluated on the basis of cell shape, microcapillary passage, ROS production, NETs formation, expression of activated CD11b and the cell viability. **Result:** HRG treatment kept the rounding shape of differentiated neutrophil-like cells, decreased the cells passage time through microcapillaries, inhibited the ROS production, NETs formation and expression of activated CD11b on the cells surface. Moreover, the cells could survive longer in presence of HRG than control groups. **Conclusion:** We showed that the ATRA-induced differentiated cell lines could be used as the alternative cells to investigate the effect of HRG on neutrophils. This method can be used for standardization tests essential for pharmaceutical development.

Oral administration of gluten induces anaphylactic reaction in mice sensitized percutaneously by various kinds of hydrolyzed wheat protein

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Hydrolyzed wheat protein (HWP) is obtained by hydrolyzing gluten, a typical allergen of wheat, which is widely used as an additive in cosmetics and food products. Recently, HWP is reported to induce IgE-mediated hypersensitivity by percutaneous sensitization and/or food ingestion of wheat protein, although it has not yet been examined if wheat or HWP can induce allergic responses after the percutaneous sensitization. In this study, we investigated whether allergic reaction was induced by repeated oral administration of gluten to mice sensitized with various kinds of HWPs. Mice were sensitized by percutaneous exposure to HWPs in the back skin for 4 weeks, and then mice were challenged orally with gluten 9 times for 3 weeks with or without aspirin for accelerating gluten absorption. A decrease in rectal temperature or anaphylactic death was not observed in the group challenged orally with gluten. In contrast, mice given oral administration of gluten with aspirin showed a significant decrease in rectal temperature leading to some cases of anaphylactic shock. These findings demonstrate that percutaneous sensitization with some HWPs and oral challenge with gluten and aspirin can induce anaphylactic responses, and indicate that some HWPs have high antigenicity by acidification and hydrolysis with heat during the manufacturing process.

Phosphodiesterase 5 inhibitor is effective both CKD and nephrotic syndrome models by protecting glomerular filtration barrier.

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Objective. Phosphodiesterase (PDE) 5 inhibitor is reported to have renoprotective effects, however, its mechanisms are unknown. In this study, we investigated the effects of a PDE5 inhibitor, tadalafil, on two renal dysfunction model. ***Materials and Methods.*** **1. CKD model.** We used Dahl salt-sensitive rats, which cause hypertension and CKD by high salt diet. They were divided into 4 groups, normal salt, high salt, tadalafil 1 mg/kg and 10 mg/kg treatment. **2. Nephrotic syndrome model.** Adriamycin(ADR) induced nephrotic model was used. We divided into 3 groups, control, ADR, and ADR+tadalafil 10 mg/kg. We evaluated kidney function and tissues in both models. ***Results.*** **1. CKD model.** High salt induced renal dysfunction and hypertension. Tadalafil 10 mg/kg treatment prevented renal dysfunction and hypertension. Tadalafil 1 mg/kg treatment also prevented kidney dysfunction, but not hypertension. Histopathological analysis revealed that tadalafil treatment attenuated glomerular injury. **2. Nephrotic syndrome model.** ADR injection induced high urinary protein. Two and 4 weeks of tadalafil treatment attenuated proteinuria. ***Conclusion.*** This study suggested that tadalafil is effective both CKD and nephrotic syndrome.

Development of nafamostat-induced hyperkalemia in rats. －A model of decreased renal excretion by a concomitant administration of amiloride－

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Nafamostat is a serine protease inhibitor and is known to cause hyperkalemia in clinical practice. Its mechanisms have been thought to inhibit sodium channels.The study aimed to develop nafamostat-induced hyperkalemia model to investigate mechanisms of the serine proteases for hyperkalemia. Nine-week-old Wistar-Imamichi male rats were used. Catheters were placed in the femoral vein, bladder, and jugular vein under sevoflurane anesthesia. Urine and blood were collected every 15 min by 8 times. (1) Nafamostat (1.2 mg/kg/h, c.i.) vs 5% Glucose groups. (2) Combination (Nafamostat 0.9, 1.8 or 3.6, c.i. and Amiloride 18.0 μg/kg, i.v. after 54.0 μg/kg/h, c.i.) vs 5% Glucose and Amiloride (18.0, i.v. after 54.0, c.i.) groups. Serum and urinary potassium were measured by the ion electrode method. (1) Changes in serum potassium level and urinary potassium excretion did not increase with nafamostat alone. (2) In the combination group, serum potassium level increased from 30 min to 90 min after administration compared with amiloride alone. Urinary potassium excretion showed a downward trend. A hyperkalemia rat model with decreased renal excretion was developed by nafamostat in combination with amiloride, which mechanism might be appearance of a potential inhibitory action of serine proteases to amiloride-sensitive sodium channels.

Age-related differences in responses to hydrogen sulfide in the bladder of spontaneously hypertensive rats

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We have confirmed that hydrogen sulfide (H_2S) is a possible relaxation factor in the rat bladder, and H_2S-induced bladder relaxation is impaired in 18-week-old (18W) spontaneously hypertensive rats (SHRs), which show bladder dysfunctions. We compared effects of NaHS and GYY4137 (H_2S donors) on bladder contractility and micturition reflex, and H_2S contents and expression of enzymes related to H_2S biosynthesis (CBS, MPST and CAT) in the bladder between 12W and 18W male SHRs. Effects of NaHS (1×10^{-8} to 3×10^{-4} M) were evaluated on carbachol (10^{-5} M)-induced pre-contracted bladder strips. Under urethane-anesthesia, effects of intravesically instilled GYY4137 (10^{-8} to 10^{-6} M) on the rat micturition reflex were examined. The H_2S contents and expression of CBS, MPST and CAT were measured by methylene blue method and western botting. Compared to 12W SHRs, NaHS-induced maximal relaxation of bladder strips was significantly decreased, GYY4137-induced intercontraction intervals prolongation was attenuated, the bladder H_2S content and expression level of CBS, MPST and CAT in the bladder dome was higher in 18W SHRs. These results indicate that H_2S-induced bladder relaxation in SHRs is impaired time-dependently, suggesting that early intervention in SHRs with H_2S donors may prevent the development of hypertension-mediated bladder dysfunctions.

Differential effects of ketamine metabolites on depression-like behaviors induced by chronic corticosterone injection in mice

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Clinical and preclinical studies have shown that the NMDA receptor antagonist ketamine exerts rapid and long-lasting antidepressant effects. Although ketamine metabolites might also have potential antidepressant properties, controversial results have been reported on (*2R,6R*)-hydroxynorketamine ((*2R,6R*)-HNK) in particular and there is little information on the effects of other ketamine metabolites. Here we aimed to compare the effects of (*R*)-norketamine ((*R*)-NK), (*S*)-NK, (*2R,6R*)-HNK and (*2S,6S*)-HNK in a mouse model of depression induced by chronic corticosterone (CORT) injection. None of these ketamine metabolites at doses up to 20 mg/kg showed antidepressant-like activity in naïve male C57BL6/J mice. Chronic CORT treatment increased immobility in the forced swim test and caused anhedonic-like behaviors in the female encounter test. A single administration of (*R*)-ketamine, but not an SSRI fluoxetine, showed antidepressant-like activity in chronic CORT-treated mice. (*S*)-NK and (*2S,6S*)-HNK dose-dependently reduced the increased immobility at 30 min after injection, while (*R*)-NK or (*2R,6R*)-HNK did not. Additionally, (*S*)-NK and (*2S,6S*)-HNK improved anhedonic-like behaviors at 24 h after injection. These results suggest that (*S*)-ketamine metabolites (*S*)-NK and (*2S,6S*)-HNK have potent acute and sustained antidepressant effects.

Noradrenergic neurons in the locus coeruleus are critical for coping with social stress in lactating female mice

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Parental stress is one of the factors to cause child abuse. However, not all of the parents cease to raise their children under severe stress conditions. The failure of stress coping may be involved in child abuse. In this study, we investigated the neuronal mechanism for stress coping using a social stress in lactating female mice. A C57BL/6J dam was housed with a strange ICR male mouse for ten minutes at postpartum day one, followed by exposure to the male overnight with a transparent separation. After repetition with the same procedures for four consecutive days using a strange male every day, the behavioral tests were performed. The repeated social stress mildly inhibited maternal care and decreased immobility in the forced swim test, indicating that dams exposed to the social stress nurture their pups with increasing active coping behavior. We next investigated whether noradrenergic neurons of the locus coeruleus (NA-LC) involved stress coping in dams. Chemogenetical inhibition of NA-LC during social stress severely prevented maternal care and increased immobility in the forced swim test. These findings indicate that NA-LC are required for coping with social stress in lactating female mice. The failure of stress coping via NA-LC may cause neglect in child care with depression.

Functional evaluation of the patient-derived iPS cells from *PDGFB*-variants in idiopathic basal ganglia calcification

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Idiopathic basal ganglia calcification (IBGC) is an intractable disease characterized by bilateral calcification in basal ganglia and other regions. The causative genes have been identified. Among them, the variant frequency of *PDGFB* in familial IBGC is about 10%. *PDGFB* encode platelet-derived growth factor B (PDGF-B). Previous studies showed PDGF-B is expressed in endothelial cells and neurons in the brain and PDGF-BB, a homodimer of PDGF-B, stimulates pericytes which are abundant in the brain and the Pi transport in the vascular smooth muscle cells. In this study, variant analyses of *PDGFB* for IBGC patients showed four novel pathogenic variants, c.160 + 2T > A, c.457 −1G > T, c.33_34delCT and c.342_343insG. The iPS cells (iPSCs) from three patients with novel *PDGFB* variant were established and endothelial cells were induced. Enzyme-linked immunoassay analysis showed that the levels of PDGF-BB in the blood sera of patients with *PDGFB* variants were decreased to 34.0% of that of the control levels. Those in the culture media of the endothelial cells derived from iPSCs of patients decreased to 58.6% of the control levels. As the endothelial cells developed from iPSCs of the patients showed a phenotype of the disease, IBGC-specific iPSCs will give us more information on the pathophysiology and the therapy of IBGC in the future.

Psychiatric-disorder-related behavioral phenotypes and cortical hyperactivity in a mouse model of 3q29 deletion syndrome

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The 3q29 microdeletion is a rare recurrent copy number variant (CNV) leading to an increased risk for neurodevelopmental disorders, such as intellectual disability and autism spectrum disorder (ASD), and a >40-fold increased risk for schizophrenia. However, the neurobiological basis for 3q29 deletion syndrome is currently unknown. In order to investigate the biological changes induced by the microdeletion, we generated a mouse model of human 3q29 deletion syndrome by deleting the orthologous region. 3q29 deletion (Df/+) mice showed reduced body and brain weight. Importantly, Df/+ mice showed deficits in social interaction and prepulse inhibition, which are reminiscent of the phenotypes in patients with 3q29 deletion syndrome. By unbiasedly analyzing the whole-brain neural activity, we found that neuronal activity was abnormally activated in a restricted region of the cortex of Df/+ mice. Furthermore, we found that the expression levels of immediate early genes were increased and that the number of parvalbumin positive neurons was decreased in the cortex of Df/+ mice. Our results suggest that Df/+ mice may provide important clues for understanding the disease-causative molecular and cellular pathology of psychiatric disorders.

1-SS-52 Student Sessions

Neuronal activity backpropagating in dentate circuit

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Hippocampal sharp waves / ripples (SPW-Rs) are high-frequency oscillations emitted mainly during slow-wave sleep or quiet rest states and play a key role in memory consolidation. While SPW-Rs are initiated in the CA3 subregion and propagate to the downstream CA1 subregion, we observed that they also propagate back to the dentate gyrus. However, neither the role of CA3-to-DG SPW-Rs backpropagation nor its propagation mechanism has been fully understood. We previously demonstrated that the subthreshold membrane potentials of hilar mossy cells reflect the activity of SPW-Rs initiated in acute brain slice preparations. We thus hypothesize that mossy cells relay CA3 SPW-Rs backward to the dentate gyrus. Using *in vitro* whole-cell current-clamp technique, we simultaneously recorded the membrane potentials of up to five mossy cells in combination with recordings of local field potentials from the CA3 *stratum pyramidale*. Information theoretical analysis revealed that the activity patterns of SPW-Rs predict the combinatorial dynamics of the membrane potentials of multiple hilar mossy cells. For further confirmation, we conducted *in vivo* whole-cell recordings from mossy cells together with recordings of local field potential of the CA1 subregion in urethane anesthetized mice. We thus concluded that mossy cells are responsive to specific patterns of ripple information at the subthreshold level. Our research approaches further elucidation of brain information dynamics and will provide a new perspective to an information processing mechanism.

Osteoblastic mTORC1 accelerates acute myeloid leukemia growth via IL-6 signaling

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Although there is increasing evidence that bone forming osteoblasts provide a microenvironment for leukemic stem cells (LSCs) and play a critical role in the maintenance and retention of LSCs, how those cells contribute to leukemia growth remains largely unclear. The mTOR complex 1 (mTORC1), a member of the serine/threonine kinases, is known to regulate the cellular function in various cell types. Using an MLL-AF9 acute myeloid leukemia (AML) mouse model, we found that AML cells enhance the mTORC1 activity in osteoblasts *in vivo* and *in vitro*. The osteoblast specific inactivation of *Tsc1*, a negative regulator of mTORC1, drives differentiation of hematopoietic stem cells (HSCs) toward myeloid lineage during steady state and promotes AML growth. Among the secretory factors examined, interleukine-6 (IL-6) was the most upregulated gene in *Tsc1*-deficient osteoblasts. Genetic inhibition of IL-6 receptor in AML cells significantly rescued tumor growth in osteoblast specific *Tsc1*-deficient mice. Collectively, our studies suggest mTORC1/IL-6 axis in osteoblastic niche could be a novel therapeutic target for AML.

1-SS-54 Student Sessions

The role of stathmin in the antiproliferative effects of eribulin in breast cancer cells

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Stathmin is a member of microtubule destabilizing proteins that modulate the dynamics of microtubule polymerization and depolymerization. Stathmin promotes microtubule depolymerization and the activity was regulated by its phosphorylation state. It has been reported that high stathmin expression is associated with poor prognosis in breast cancer patients. Eribulin, an analogue of the marine natural product halichondrin B, is a microtubule-depolymerizing drug that has been used in the treatment of patients with advanced breast cancer. In this study, we examined the involvement of stathmin in the antiproliferative activities of eribulin in breast cancer cells (MCF7 and MDA-MB-231). Eribulin induced phosphorylation of stathmin in both cells. The eribulin-mediated phosphorylation of stathmin was attenuated by the inhibitors of protein kinase A (H89) and Ca^{2+}/calmodulin-dependent kinase II (KN62). In addition, the phosphorylated stathmin was reduced by the protein phosphatase PP2A activator FTY720, whereas it was increased by the PP2A inhibitor okadaic acid. Of note, eribulin did not directly affect the phosphatase activity of recombinant PP2A, but the expression of PP2A subunits was reduced in eribulin-treated cells. Furthermore, the antiproliferative effect of eribulin was more efficient in stathmin-overexpressing cells compared to control. Together these data provide a novel mechanism of antiproliferative effects of eribulin which is mediated through stathmin dynamics in breast cancer cells.

Suppression of LAT1 in endothelial cells of tumor tissues exhibits an anti-angiogenesis effect

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Angiogenesis plays a critical role in supporting tumor growth and metastasis. L-type amino acid transporter 1 (LAT1), a transporter for large neutral amino acids, is expressed at a high level in a wide range of cancers. LAT1 has, thus, been proposed as a promising molecular target for cancer therapy. We have recently found that, in addition to the cancer cells, LAT1 is also highly expressed in endothelial cells of human pancreatic cancer tissues and xenograft tumor models. In *ex* and *in vivo* angiogenesis assays, we found that pharmacological inhibition and genetic ablation of endothelial LAT1 exert an anti-angiogenic effect. Moreover, contribution of LAT1 in angiogenesis was verified in *in vitro* angiogenesis assays using human umbilical vein endothelial cells. As a possible mechanism underlying the upregulation of LAT1 in endothelial cells, we found that angiogenic growth factors, VEGF and FGF2, induce LAT1 expression. These results indicate that the enhanced amino acid uptake mediated by LAT1 in tumor-associated endothelial cells contributes to tumor angiogenesis. Beside the previously well-recognized inhibition of amino acids uptake of LAT1 in cancer cells, suppression of the tumor angiogenesis via the inhibition of endothelial LAT1 would therefore also contributes to the anti-tumor effect of cancer therapies targeting LAT1.

1-SS-56 Student Sessions

Evaluation of cytotoxicity of bispecific antibody against mesothelioma

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Malignant mesothelioma is a fatal tumor caused by past exposure to asbestos. In Japan, mesothelioma due to asbestos exposure is a major public health problem. The prognosis for mesothelioma patients is very poor. Satisfactory recovery is often not possible with chemotherapy and/or radiotherapy. Therefore, a new effective anti-mesothelioma drug is urgently required. In previous study, we isolated a highly specific anti-mesothelioma mAb, SKM9-2. The specificity and sensitivity of SKM9-2 to mesothelioma reach 99% and 92%, respectively. SKM9-2 recognizes the sialylated protein HEG homolog 1 (HEG1), a novel mucin-like membrane protein.

In this study, we investigated the cytotoxic activity of bispecific antibodies (bsAbs) in which the antigen-recognition domains of SKM9-2 and anti-CD3 (OKT3) were linked. BsAbs were purified from the culture supernatant of stably expressed mammalian cell lines and were analyzed about the binding to SKM9-2 epitope and CD3. Some bsAbs strongly bound to SKM9-2 epitope but not CD3. In these bsAbs, two antigen recognition domains may interfere. A bsAb that bound to both SKM9-2 epitope and CD3 showed a strong T cell-dependent cytotoxicity against mesothelioma.

SKM9-2 binds to mesothelioma cells but not normal tissues. Thus, T cell-engaging bsAb including SKM9-2 may be a promising drug for mesothelioma.

Doxorubicin-caused cardiomyopathy is due to an inhibition of the fusion step on the autophagy flux

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Doxorubicin (Dox), widely used anti-tumor agent, damages to the various molecules and organelle in the heart by augmenting the oxidative stress, leading to induction of severe cardiomyopathy, while the mechanism of Dox-caused cardiomyopathy remains uncertain. Autophagy is a process that degrades the intracellular accumulated molecules in the lysosome to maintain cellular homeostasis. We have reported that Dox activated the autophagy flux, but caused cell death in a rat myoblast cell line, H9c2 cells used as a model for Dox-induced cardiomyopathy. To further elucidate the mechanism of Dox-caused cardiomyopathy, we first used several cell death inhibitors in the presence of Dox. 3-methyadenine, an autophagy inhibitor, showed a suppress of Dox-induced cell death indicating that autophagy is a primary cause of cell death by Dox. We next evaluated the effect of Dox on the autophagy process and found that it inhibits the fusion step of autophagosome with lysosome to form autolysosome. Since the various membrane trafficking molecules are crucial to regulate the fusion step, we further examined the effect of Dox on the expression of protein X that is potentially control autophagy. As the expression level of protein X was significantly decreased by Dox, we knocked-down the protein X to confirm the role of the protein. Protein X-knockdown cells showed the impairment of autolysosome formation and autophagy flux. These results suggest that Dox-caused the decrease in the expression of protein X resulted in inhibiting the fusion step of autophagosome with lysosome on the autophagy flux.

Anti-tumor activity and adverse effects of a novel compound with high specificity to tetraplex DNA

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Telomerase which extends telomere sequences to the end of chromosome is overexpressed in more than 80% of cancer cells, thus it is expected as a target for developing anticancer drug. As the telomeric repeat sequences form characteristic quadruplex DNA structure, we here investigated the effects of novel compounds with improved binding specificity to quadruplex DNA structure, namely, cyclic naphthalene diimide (cNDI) and cyclic anthraquinone (cAQ), on various cultured cells.

Human cancer cell line Ca9-22, SAS, HSC-2, HeLa, and human normal keratinocytes, mouse bone marrow cells were used in this study. Cell proliferation was examined by WST-8 assay and direct counting using a hemocytometer. Gene expression was analyzed by general PCR and real-time PCR using reverse transcribed total RNA prepared from the cells.

All the derivatives of cNDI and cAQ inhibited cell growth in a dose-dependent manner, and the effect of some compounds tended to correlate with the mRNA expression level of TERT gene. Furthermore, the novel compounds showed a strong cell growth inhibitory effect against cancer-derived cell lines by comparing mouse bone marrow cells with human normal epidermal keratinocytes. The results suggested that cNDI and cAQ derivatives are considered to be promising as new anticancer agents with improved cancer specificity.

The role of the novel sex steroid membrane receptor mPRε on metabolic homeostasis.

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Progesterone is a sex steroid hormone synthesized by the ovary, and plays a pivotal role for reproductive functions such as ovulation and the maintenance of pregnancy. Although these effects of progesterone have been implicated in nuclear progesterone receptors (PRs)-mediated classical signaling pathway, key pathways involved in non-classical progesterone signaling has been provided by the identification of membrane progesterone receptors (mPRα, mPRβ, mPRγ, mPRδ, and mPRε). Interestingly, mPRs may have been related to progesterone-mediated unknown rapid non-genomic action that cannot be currently explained by their genomic action through PRs. However, the structure, intracellular signaling, and physiological functions of these progesterone receptors are still unclear. In this study, we confirmed that mPRε, among mPRs receptors, is specifically expressed in the white adipose tissue (WAT), liver, and kidney of adult male and female mice. Furthermore, progesterone- mPRε signaling may contribute to suppression of glucose uptake and impair glucose tolerance in WAT. These findings provide new insights of regarding the non-genomic action of progesterone in metabolic homeostasis and novel therapeutic targets and strategies for metabolic disorder such as obesity and type 2 diabetes mellitus.

Development of a novel chemical tag tool for calcium imaging by near-infrared fluorescence

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Ca^{2+} plays important roles as a second messenger in a wide range of biological phenomena including neurotransmission. Near infrared (NIR) fluorescence is suitable for *in vivo* Ca^{2+} imaging because of its high tissue penetration, low light scattering, and minimal autofluorescence. Many types of NIR chemical probes have been developed but they lack selectivity for labeling of particular cell types upon multi-cell bolus loading. We developed DeQODE chemical tag system as a new chemical biology tool, in which a small-molecular QODE probe can visualize Ca^{2+} signals with NIR fluorescence selectively inside the target cells expressing DeQODE tag. In an application of our DeQODE tag system to primary cultures of rat hippocampal neurons, neurons expressing DeQODE tag were selectively labeled by QODE probe. We successfully visualized Ca^{2+} signals of the target neurons in response to electrical stimulation at 10 Hz. We will perform *ex vivo* and *in vivo* application of our chemical tag system.

Role of a mechanosensitive ion channel PIEZO1 in muscle satellite cells

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Muscle-resident stem cells called muscle satellite cells (MuSC) play an essential role in muscle regeneration. Mechanosensation is presumed to be critical for activation of MuSCs, but the molecular entity that determines the cell fate in MuSCs through converting the mechanical stimuli into biochemical signals remains to be elucidated. Here we identify PIEZO1, a mechanosensitive ion channel that is activated by membrane tension, as a critical determinant for activation of MuSCs. *In silico* analysis demonstrates that PIEZO1 is predominantly expressed in MuSCs but not in mature myofibers. By utilizing *Piezo1-tdTomato* mice where endogenous PIEZO1 is fused with a fluorescent protein tdTomato, our immunofluorescent analysis reveals that PIEZO1 is accumulated to the cleavage furrow during cell division of MuSCs. Moreover, a conditional deletion of *Piezo1* leads to delayed myofibers regeneration after cardiotoxin-induced myofiber injury, at least in part due to the cell division delay in MuSCs. Thus, our results indicate that PIEZO1 is a bona fide mechanosensor whose ion channel activity is required for completion of cell division in MuSCs.

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Clustering therapeutic drugs using FDA Adverse Event Reporting System

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Therapeutic drugs have been classified based on pharmacodynamics and disease indications. However, it has gradually been revealed that profiling of side effects can be used to classify therapeutic drugs and to find novel disease indications of drugs. In this study, we generated multidimensional vectors for each therapeutic drug based on the cosine similarity of indications or side effects in the US FDA Adverse Event Reporting System (FAERS). Using the spatial density, the multidimensional vectors were clustered based on the indications and side effects in FAERS. By comparing these clusters, we were able to identify several sets of therapeutic drugs that were common in the two clusterings, including a few sets comprising of therapeutic drugs with different pharmacodynamics and different disease indications. These findings suggest that clustering therapeutic drugs based on similarities of indications and side effects reported in public databases can be useful to find new functions of therapeutic drugs.

Effect of Piezo 1, a mechanoreceptor, on intraocular pressure and trabecular meshwork

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In primary open angle glaucoma patients, inhibition of aqueous humor discharge due to accumulation of extracellular matrix (ECM) in trabecular meshwork (TM) is often observed. It is known as a cause of high intraocular pressure (IOP). A previous report showed that TM cells express 11 types of mechanoreceptor including Piezo 1. However, its role in TM cell is still unclear and this study has evaluated the effect of Piezo 1 on ECM expression level in TM. At first, we measured the IOP in mice treated with a Piezo 1 agonist, Yoda 1, and observed a drop in IOP. Fibronectin expression level in the TM after Yoda 1 treatment in mice was evaluated by immunostaining, and they were both decreased. Then, by Western blotting analysis, we evaluated cPLA2 phosphorylation, MMP-2 expression and fibronectin expression using human cultured TM cells. Yoda 1 increased phosphorylated-cPLA2 and MMP-2 expression, and suppressed fibronectin expression in TM cells. These results suggest that Piezo 1 activation in TM may be responsible for suppressing fibronectin expression through cPLA2 activation, and one of new therapeutic targets for glaucoma.

Time-course analysis of the changes in intracellular amino acid concentrations and amino acid-related signaling pathways in cancer cells induced by the inhibition of cancer-type amino acid transporter LAT1

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There is an increased demand for nutrients in cancer cells compared to normal cells. Accordingly, the nutrient intake and metabolism in cancer cells are regarded as promising therapeutic targets. L-type amino acid transporter 1 (LAT1) is upregulated in cancer cells of various tissue origin, and has a large effect on the uptake of amino acids. LAT1 inhibitors have been shown to suppress cell proliferation and tumor growth *in vitro* and *in vivo*. However, it has not been clarified yet how the intracellular concentration of each amino acid changes and, concomitantly, what cellular responses are induced in cancer cells when treated with LAT1 inhibitors. In this study, we established a high performance liquid chromatography (HPLC) system to quantify amino acids. Using the system, we could successfully follow the time course of the change in the concentration of cellular amino acids in human pancreatic cancer MIA PaCa-2 cells treated with LAT1 inhibitor. In the same experimental condition, we also detected changes in the amino acid-related signaling pathways including mTORC1 pathway and autophagy. Currently, we are monitoring the changes in the amino acid concentrations in the cell culture medium. The detailed effects of LAT1 inhibitors on the intra- and extracellular amino acids, and the subsequent cellular responses will be discussed.