1-SS-01

Roles of macromolecular complexes in calcium-sensitivity of the cardiac I_{Ks} channel

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Roles of macromolecular complexes in calcium-sensitivity of the cardiac I_{Ks} channel

Cardiac repolarization process is regulated by the slow component of the delayed rectifier potassium (I_{Ks}) channel composed of Ca^{2+}-sensitive KCNQ1 and KCNE1. Although calmodulin and AKAP9 regulate I_{Ks} channel for instance, the entire macromolecular system has not been elucidated. Thus, to seek macromolecular complexes comprehensively, we performed proteomics analysis using a transgenic mouse overexpressing KCNQ1-KCNE1 fusion protein specifically in the heart. We identified 163 proteins as potential I_{Ks} binding partners by immunoprecipitation using a goat anti-KCNQ1 antibody. Pathway analysis of these proteins revealed that the most significant biofunction is the Calcium Signaling including 16 molecules. To understand how the protein-protein interactions affect Ca^{2+}-dependent I_{Ks} regulation, we picked up the cardiac Na^{+}-Ca^{2+} exchange transporter (NCX1). Pull-down assays in dog ventricles with purified GST-fusion proteins of full-length or parts of KCNQ1 identified that the N- and a proximal C-terminus of KCNQ1 contribute the interaction. These results imply that the Ca^{2+}-sensitivity of I_{Ks} channel is regulated by the interaction between the I_{Ks} channel and NCX1. Pharmacological experiments will be performed to pursue its mechanistic insights.

1-SS-02

Critical moieties of aromatic amino acids for the interaction with organic anion transporter OAT1: Implications for reducing the renal background in tumor imaging

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The excellent tumor selectivity of amino acid-based imaging probes, [^{18}F]FAMT (3-[^{18}F]fluoro-a-methyl-l-tyrosine) and [^{123}I]IMT (3-[^{123}I]iodo-a-methyl-l-tyrosine), is supported by their selectivity for LAT1 (L-type amino acid transporter 1) which is specifically expressed in tumor cells. However, FAMT and IMT show a physiological background in kidney. Their renal accumulation is suppressed by probenecid, suggesting a contribution of organic anion transporter OAT1. To reveal the critical moieties responsible for the interaction with OAT1, we performed a structure-activity relationship analysis of aromatic amino acid derivatives *in vitro*. We revealed that both halogen and hydroxyl groups on the benzene ring are critical for the interaction with OAT1. Their positions on the benzene ring also affected the interaction. In contrast, the a-methyl moiety, which is essential for the selectivity to LAT1, was dispensable for the interaction with OAT1. These results hold a significant implication for designing not only the tumor-specific imaging probes with less renal background, but also the therapeutic agents for targeted radionuclide therapy with less adverse renal damage.

1-SS-03

The involvement of EAAC1 in diurnal variation of ischemic Zn^{2+} toxicity

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[Aim] It was reported that temporal changes in severity of ischemic brain injury, but the mechanism is mostly unknown. In ischemic hippocampus, extracellular Zn^{2+} accumulates in neurons, resulting in neuron death. Excitatory amino acid carrier (EAAC) 1 reduces zinc toxicity. Here, we examined the involvement of EAAC1 in temporal changes in ischemic zinc toxicity.
[Methods] Mice (12 weeks) were subjected to ischemia at 09:00 (ZT4) or 23:00 (ZT18). At 72 h after, zinc accumulation was assessed by Zn^{2+} probe, TSQ, and the number of neurons were examined by immunostaining. Diurnal changes in hippocampal EAAC1 expression were assessed by western blot. Mice were subjected to ischemia at ZT18 after an EAAC1 inhibitor, TBOA, injection (i.c.v.) and we examined zinc accumulation and the number of neurons.
[Results] Ischemia induced TSQ(+) cells in hippocampus and the number of TSQ(+) cells were less at ZT18 than ZT4. Compared to ZT4, a decrease in neuron death were observed at ZT18. EAAC1 expression was higher at ZT18 than ZT4. Besides, TBOA increased TSQ(+) cells and decreased neuron death at ZT18.
[Conclusion] These results suggest temporal changes in severity of ischemic neuronal damage might be mediated by zinc accumulation via diurnal variation of EAAC1 expression.

1-SS-04

Molecular mechanism of substrate recognition by Leucine-Specific Binding Protein

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In *Escherichia coli*, the active transport of branched-chain amino acids was performed by three different kinetically system: Leucine-isoleucine-valine (LIV)- I, II and Leucine-specific (LS) system. The transport capacity of LS system depends on a periplasmic protein, leucine-specific binding protein (LS-BP). In previous studies, the substrate specificity of LS-BP was revealed *in vivo*, but the mechanism of substrate recognition remains unclear. In this study, we purified LS-BP and measured the affinity for leucine and its derivatives by BIACORE. Since the affinity of LS-BP for leucine and its derivatives exceeded the maximum range of BIACORE, the Km value could not be determined. Then we developed an *in vitro* assay to investigate the substrate recognition of LS-BP by using radiolabeled leucine. In this assay, the derivatives modified with NH_2, COO, or Cγ did not show an obvious binding to LS-BP, while no significant differences were observed between leucine and its derivatives modified with OH, Cα or Cβ. Those results suggest that LS-BP recognizes NH_2, COO, and Cγ of leucine.

1-SS-05

Pathologic mechanism of ferric chloride-induced pulmonary fibrosis

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Idiopathic pulmonary fibrosis(IPF) is considered a fatal respiratory disease. However, a large number of anti-fibrotic drugs described in the current experimental models including bleomycin(BLM)-induced fibrosis have not been translated into clinical practice successfully, suggesting that a new pulmonary fibrosis model mimicking most of pathological features of human IPF is needed. We established a new pulmonary fibrosis mouse model by injecting $FeCl_3$ solution into the left upper lobe central part. In our lung lobe-specific fibrosis model, fibrogenesis was progressive and irreversible, the feature of which mimics human IPF and cannot be observed in other lung fibrosis models. Here, we investigated how ferric chloride could induce pulmonary fibrosis.

At 10 days post-injury with ferric chloride, severe fibrosis of the whole lobe was observed. We temporally and spatially followed the injury process of the lung by monitoring the indices such as ferrous iron accumulation, production of Reactive oxygen species , endoplasmic reticulum stress and apoptosis. We also performed a comprehensive analysis of microRNAs expression. We will discuss the molecular mechanisms underlying pulmonary fibrosis induced by ferric chloride, compared with BLM-induced fibrosis model.

1-SS-06

Characterization of itch-related scratching behavior in chronic atopic dermatitis in hairless mice

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Itch is the most troublesome symptom in atopic dermatitis (AD). Although skin barrier dysfunctions and aberrant immune responses are thought to contribute to AD itch, the precise mechanism is yet to be clarified. In this study, we newly established a chronic AD mouse model to identify the mechanism of itch in AD. Hairless mice were fed a special diet deficient in polyunsaturated fatty acids and starch to induce dry skin with barrier dysfunction (dry-skin mice). Ointments containing a crude extract of house-dust mite were then repeatedly applied to the skin of the dry-skin mice. The dry-skin mice treated with a mite extract (DM mice) exhibited AD-like skin manifestations and histology. DM mice showed robust scratching behavior, which was partially attenuated by treatment with either betamethasone or tacrolimus, but not by that with olopatadine. Genome-wide gene expression analysis revealed that DM mice had a similar skin gene expression profile to that of human AD. Furthermore, increased expressions of *Chi3l3, Chi3l4* and *Ear11* in DM mice were consistent with exacerbation of scratching behavior. Thus, we will further examine the role of proteins encoded by these genes in itch-related behavior in this AD model.

1-SS-07

Increase in Thermo-sensitive TRP Channel-Expressing Neurons in Acid Reflux Esophagitis Model Rats

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TRPV1 expressing on sensory neurons in mouse lower esophageal sphincter (LES) is considered as association with heartburn symptoms in patients with acid reflux esophagitis (RE). In the present study, we examined changes in TRPV1 and TRPM8 expressions in LES in RE model rats. RE was produced by wrapping duodenum with Nelaton catheter, and ligating the transitional zone between the forestomach and the glandular portion with silk thread under anesthesia. TRP channel and neuronal markers in rat LES were detected by using immunohistochemical staining. In normal rats, numerous TRPV1 nerve fibers were detected in mucosal and submucosal layers. TRPM8-expressing cell bodies were observed in myenteric plexus. In double labeling studies, TRPV1 partly colocalized with calcitonin gene related peptide (CGRP), substance P and neuronal nitric oxide synthase (nNOS) on nerve fibers. In RE model rats, TRPV1-expressing nerve fibers were increased mainly in muscle and mucosal layers. Moreover, TRPM8-expressing cell bodies were increased in myenteric plexus. In conclusion, we suggest that increased TRPV1-expressing nerve fibers and TRPM8-expressing cell bodies were found in myenteric plexus in RE model rats, which might be responsible for heartburn in RE.□

1-SS-08

Establishment of low grade inflammatory bowel disease model mice and anti-inflammatory effect of choline esterase inhibitor

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Low-grade inflammation persists in many patients with clinically quiescent inflammatory bowel disease (IBD). The current study aimed to establish low-grade IBD model mice. In addition, the anti-inflammatory effect of the cholinesterase inhibitor neostigmine was also investigated in this model. C57BL/6J mice were used. Colitis was induced by the addition of 0.1–3%(w/v) dextran sulfate sodium (DSS) to drinking water for 7 days. Following 3% DSS treatment, weight loss, appearance of bloody stool and changes in stool quality were evident by day 4 and peaked at day 7. DSS at 1% elicited low-grade inflammation in the colonic mucosa and increased myeloperoxidase (MPO) activity. In immunohistochemical study, increased MPO-immunopositive neutrophils were observed in the colonic mucosa of low-grade colitis model. Neostigmine dose-dependently inhibited the increase of MPO activity. The α7-nicotinic receptor antagonist partly reversed anti-inflammatory effect of neostigmine. In conclusion, we suggest that low-grade IBD model mice are established by using 1% DSS-containing drinking water, and neostigmine provides anti-inflammatory effect through the stimulation of α7-nicotinic receptors in this model.

1-SS-09

Flurbiprofen may ameliorate diabetes by reducing endoplasmic reticulum stress

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Reducing pancreatic β cell failure may suppress the onset and progression of type 2 diabetes. Endoplasmic reticulum stress (ER stress) has been reported to be involved in the onset of metabolic diseases such as diabetes and obesity. We previously shown that flurbiprofen, a nonsteroidal anti-inflammatory drug, may have chaperone activity and can suppress ER stress. In this study, we investigated possibility that flurbiprofen may ameliorate diabetes through reducing ER stress-induced pancreatic β cell death. We found that flurbiprofen suppressed the ER stress-induced expression of C/EBP homologous protein (CHOP), an apoptotic transcription factor, in mouse pancreatic β cells (Min6 cell line). Additionally, flurbiprofen suppressed ATF4, an upstream regulator of CHOP, suggesting that flurbiprofen may protect β cells by suppressing apoptosis through regulating ATF4-CHOP pathway. Furthermore, we found that flurbiprofen reduced blood glucose levels, and increased pancreatic and serum insulin levels without affecting body weight in db/db diabetic mice model. We are now performing comprehensive analysis using microarrays to further elucidate pharmacological action of flurbiprofen in pancreatic β cells. Overall, flurbiprofen may be able to ameliorate diabetes by reducing ER stress in β cells.

1-SS-10

DGKδ deficiency in mouse pancreatic β-cells alleviates STZ-induced diabetes.

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Diacylglycerol kinase (DGK) phosphorylates diacylglycerol (DAG) to phosphatidic acid. We have previously demonstrated that DGKδ acts as a negative regulator of β-cell proliferation. However, it is still unknown whether DGKδ deficiency in pancreatic β-cells alleviates diabetes. In the present study, we investigated the effect of β-cell specific DGKδ deficiency on hyperglycemia in streptozotocin (STZ)-induced diabetic mice. We administered STZ to 5-wk-old mice and performed measurement of body weight and blood glucose level for 60 days. At 11 days after STZ administration, there was no significant difference in serum insulin concentrations, pancreatic insulin content, or β-cell area between β-cell specific DGKδ knockout (βDGKδKO) and control mice. At 60 days, in contrast, blood glucose level was significantly lower and serum insulin level and pancreatic insulin content were significantly higher in βDGKδKO mice than in control mice. In morphological analysis, a significant increase in β-cell area was observed in βDGKδheteroKO mice compared with control mice. These results suggest that DGKδ deficiency in pancreatic β-cells prevents STZ-induced hyperglycemia, which is likely due to maintained β-cell mass resulting from promoted β-cell proliferation.

1-SS-11

The effect of a bioactive natural product in a mouse model of diabetic neuropathy

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Diabetic neuropathy (DN) is one of the most common complications of diabetes, and has a prevalence of as high as 50% in diabetic patients. Allodynia and hyperalgesia are early symptoms of the disease. Sensory paralysis is a late symptom, and is frequently accompanied by a poor prognosis, including limb amputation. However, there is currently no effective treatment for DN. In the present study, we evaluated the effect of a bioactive natural product in a mouse model of DN produced by injection of 200 mg/kg streptozotocin (STZ). The bioactive natural product, 30 mg/kg, was administered to the diabetic mice for a period of 3 weeks, starting 1 week after the STZ injection. The behavioral effects of the bioactive natural product were evaluated using the von Frey test (to assess mechanical allodynia) and the hot plate test (to assess thermal hyperalgesia). Furthermore, we examined conduction velocity and blood flow in the sciatic nerve. Treatment with the bioactive natural product ameliorated the allodynia and hyperalgesia, and it increased conduction velocity and blood flow in the sciatic nerve in the diabetic mice with DN. These findings suggest that the bioactive natural product has therapeutic potential for the treatment of DN.

1-SS-12

Effects of Morin and Quercetin on endothelial dysfunction in diabetic mice

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Endothelial dysfunction is a key factor in development of diabetic vascular complications. We and others reported that vegetable polyphenol morin (MO) and quercetin (QC) induced vascular endothelial relaxations through the Akt/eNOS signaling pathway. However, the relaxation pathway was fully unknown. In this study, we examined about the upstream of the Akt/eNOS signaling in diabetes. In aortas isolated from diabetic (DM) or control mice, MO and QC induced dose-dependent relaxation responses. Especially, DM aortas showed enhanced MO-induced relaxation responses relative to controls. The relaxation responses with MO were not significant in the presence of each PI3K inhibitor and AMPK inhibitor. Meanwhile, the relaxation responses with QC were attenuated in the presence of PI3K inhibitor. In the presence of AMPK inhibitor, the relaxation responses with QC were attenuated in DM but there were not significant in control. In this study, we showed that QC induced NO-dependent relaxation responses via PI3K/Akt/eNOS signaling pathway in control, notably AMPK/Akt/eNOS signaling in DM. Thus, it was suggested the possibility that MO and QC have different mechanism in relaxation, and that MO had the beneficial effect on the diabetes induced endothelial dysfunction.

1-SS-13

GLP-1 improves diabetic vascular endothelial dysfunction by suppressing GRK2 activity

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Abnormal G-protein-coupled receptor kinase 2 (GRK2) accumulation has a crucial role in the development of insulin resistance and diabetes. Previous report showed that impaired insulin-induced relaxation in diabetes is improved by suppressing the GRK2 levels. Glucagon-like peptide-1 (GLP-1) is a gut hormone that promote insulin secretion. However, it is unknown whether GLP-1 directly affects diabetic endothelial dysfunction, especially GRK2 signaling. In this study, we investigated the relationship between GLP-1 and GRK2 under insulin stimulation for endothelial dysfunction. GLP-1 increased the impaired insulin-induced NO-dependent relaxation responses in diabetes. However, these responses are disappeared by treatment of Exendin-9, a GLP-1 receptor antagonist. Furthermore, phosphorylation levels of Akt and eNOS were higher in diabetes under GLP-1/insulin stimulation than non-stimulation. Additionally, in diabetes GRK2 activity was inhibited under GLP -1/insulin stimulation compared with non-stimulation, although GRK2 expression was not altered between the two groups. Those results suggest that in diabetes, GLP-1 improves the endothelial dysfunction by suppressing of GRK2 activity, which might provide a therapeutic target for diabetic vascular complications.

1-SS-14

The role of RNA granules as a platform to spatially regulate MAPK signaling

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Protein kinase C (PKC) family plays crucial roles in a wide variety of cellular functions and dysregulation of PKC signaling leads to pathophysiological states including cancer and neurodegenerative disease. PKC activity is rigorously fine-tuned by multiple mechanisms, but the spatial regulatory mechanism of PKC signaling remains fully understood. Here, we have shown that RNA granules, widely conserved non-membranous cytoplasmic structures composed of RNA-protein complexes, play a key role in spatially regulating PKC and its downstream MAPK signaling activation. Upon heat stress, PKC and downstream MAPK signaling activation was induced, which stimulates recruitment of the PKC homologue in fission yeast, from the plasma membrane into RNA granules. Intriguingly, Inhibition of the downstream MAPK signaling impaired PKC translocation to the RNA granules, whereas the constitutively active MAPKK stimulated PKC translocation to the RNA granules. We also demonstrated that the kinase activity of PKC influenced its intracellular distribution from the cell membranes to the RNA granules. Our data is the first demonstration that PKC translocation into RNA granules is a novel feedback mechanism mediated by MAPK signaling.

1-SS-15

Enterococcus faecalis extracts FK-23 modulates Propionibacterium acnes-induced lipogenesis in human sebocytes SZ-95 cells

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It is generally accepted that Propionibacterium acnes (P. Acne) is involved in the development of acne, while the mechanisms of sebaceous lipogenesis and its control is unclear. Enterococcus faecalis FK-23 had shown to promote an anti-inflammatory action in several animal models. In the current study, we examined whether FK-23 modulate lipogenesis in sebocytes. FK-23 stimulated lipogenesis, while inhibited them in the presence of P. Acne. FK-23 acutely inhibited acetyl-CoA carboxylase (ACC) phosphorylation levels, while stimulated them with P. Acne. FK-23 stimulated PPARγ expression and activity, while inhibited them with P. Acne as pioglitazone did. These combined evidences demonstrated that the dual action by FK-23 on lipogenesis should reflect differentiation machinery prior to PPARγ, leading to producing adequate levels of sebum.

1-SS-16

Nε-(carboxymethyl) lysine represses hair follicle formation by inhibiting Sonic hedgehog expression in a NF-κB-independent manner

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Nε-(carboxymethyl) lysine (CML), an advanced glycation end product (AGE), is an aging factor produced by glycation of protein. Higher levels of AGE in skin tissue are related to skin elasticity, but how CML that has accumulated in the skin affects hair follicle formation is unclear. This study constructed a simple model that mimics accumulated glycation from feeding by intradermally injecting Nε-(carboxymethyl) lysine (CML), and examined the effects on the morphogenesis of hair follicles (HF). The results showed weakening of the hair shaft and HF formation by CML. The in vitro inhibitory effect of CML on wound healing of dermal papilla cells (DPC) suggested that the mechanism influences the proliferation and migration of DPC, which are essential for HF morphogenesis. In addition, CML in DPC inhibited the expression of sonic hedgehog (Shh), a factor of tissue morphogenesis, in a NF-kB-independent manner. The findings suggest that the delay in HF formation was due to CML inhibiting proliferation and migration in DPC by inhibiting Shh expression.

1-SS-17

Effects of amino acid availability on the intracellular free amino acid concentration

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Amino acids in cells are used not only as building blocks for protein synthesis but also for other purposes such as signal transduction and ATP synthesis. For example, amino acids are essential signaling molecules to activate mTORC1 (mechanistic target of rapamycin complex 1), a serine/threonine kinase complex, which plays a key role in the regulation various biological processes including lipid synthesis, translation, autophagy and so on. Also, dysregulation of amino acid homeostasis is often implicated in the pathogenesis of cancer, diabetes and neurodegenerative disorder. Amino acids in cell culture medium are supposed to significantly affect the intracellular amino acid contents, and subsequently, amino acid-related cellular functions. However, the relationship between amino acid availability and intracellular amino acid concentration is still poorly understood. In this study, we established a reversed-phase HPLC method to quantitatively analyze the amino acids in cell lysates. Among 20 proteinogenic amino acids, 19 amino acids except tryptophan was successfully detected. By manipulating the amino acid availability in mammalian cell culture, the effects on the intracellular free amino acid concentration were further studied.

1-SS-18

Mechanisms of DCEBIO's myogenic and hypertrophic effect on C2C12 cells

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Skeletal muscle atrophy impairs quality of life. However, there is no effective medications. To maintain muscle mass, it is crucial to enhance differentiation of skeletal muscle stem cells and protein synthesis in myofibers. Our search using C2C12 cells determined that DCEBIO, a small/intermediate conductance Ca^{2+} activated K^{+} (SK/IK) channel opener promotes myogenic differentiation and muscle hypertrophy. DCEBIO hyperpolarized myoblast membrane potential and increased intracellular Ca^{2+} concentration. The expression of myogenin was elevated. DCEBIO treatment increased phosphorylation level of S6K, but not that of Akt. DCEBIO did not alter the expression of atrophy and hypertrophy related genes. SK/IK channel blocker, apamin (100 nM) and TRAM-34 (1 μM) inhibited myogenic differentiation but did not attenuate hypertrophic effect of DCEBIO in C2C12 cells. These results suggest that DCEBIO enhances myogenic differentiation by opening of SK/IK channel and activating myogenic regulatory factor. In contrast, DCEBIO increases muscle mass by activating S6K independent of SK/IK channel activation. This is a significant study for future drug development for muscle atrophy.

1-SS-19

Social defeat stress as an inducer of extracellular signal-regulated kinase activation in leptomeninges

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Social defeat stress develops depressive-like behaviors in mice by inducing inflammation both in the central nervous system and in the systemic circulation. Recent evidences have shown that the disruption of blood brain barrier leads to stress-induced behavioral alterations, suggesting that the integrity and deterioration of barriers surrounding the brain parenchyma is critical in stress-related pathophysiology. Although there has been emerging evidence showing the biochemical difference between the CSF of patients of depression and that of healthy controls, the effects of stress on the CSF system have rarely been studied. In the present study, we examined effects of social defeat stress on leptomeninges, which serve as barriers separating the brain parenchyma from the CSF and as regulators of immune reactions. We found an evidence of immediate phosphorylation of extracellular signal-regulated kinase (ERK) in the leptomeninges following a single episode of social defeat stress. This activation of ERK was diminished after repeated exposure to daily stress for 10 days. These evidences suggest that the leptomeninges are activated following an acute stress and diminish their responsiveness during the course of chronic stress.

1-SS-20

Involvement of glutamate receptors in the impairment of social behaviors induced by social defeat stress exposure as juveniles

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Glutamatergic systems play a critical role in the pathophysiology and treatment of stress-related disorders. In the present study, we conducted behavioral and neurochemical experiments to reveal involvement of glutamate receptors in the impairment of social behaviors induced by stress exposure as juveniles. Acute administration of ketamine, a non-competitive NMDA receptor antagonist and subsequent AMPA receptor stimulation attenuated the impairment of social behaviors in adolescent mice exposed to social defeat stress as juveniles. NBQX, a selective AMPA receptor antagonist prevented the attenuating effect of ketamine on the impairment of social behaviors. Although there were no significant changes in the ratios of phosphorylated protein of some NMDA subunits, that of AMPA receptor GluA1 subunit was significantly increased in the hippocampus of non-tested, defeated mice. In non-tested, defeated mice, ketamine increased the hippocampal total protein level, but not the ratio of phosphorylated protein of GluA1. These results suggest that exposure to social defeat stress as juveniles induces the impairment of social behaviors in adolescents through the functional changes in AMPA receptors.

1-SS-21

Elucidating neuronal projections from the medial prefrontal cortex responsible for the resilience to social defeat stress in mice

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Stress caused by aversive stimuli, if not excessive, is thought to provoke adaptive biological responses in rodents and primates. We have previously shown that single social defeat stress in mice activates dopamine D1 receptor in excitatory neurons of the medial prefrontal cortex (mPFC), leading to dendritic hypertrophy of these neurons and strengthening stress resilience. However, it remains elusive which brain regions mediate the action of the mPFC for stress resilience. In the present study, using c-Fos immunohistochemistry, we examined neuronal responses to single social defeat stress in multiple brain regions of adult male C57BL/6 mice. We found that the stress activated neurons in several subcortical brain regions, such as the bed nucleus of the stria terminalis (BNST), lateral septal nucleus and amygdala nuclei, which receive projections from the mPFC. We are currently exploring roles of mPFC projections to these brain areas in stress resilience by manipulating the activities of these projections using chemogenetics. Our preliminary finding points to the potential role of mPFC projection to some of these brain areas. Thus, our study paves the way for the notion that dopamine D1 receptor signaling in the mPFC coordinates its projections to subcortical areas upon short-term stress, thereby facilitating stress resilience.

1-SS-22

Involvement of nicotinic acetylcholine receptor-signaling in the impairment of social behavior in the stressed mice

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[Purpose] We investigated the effect of (-)-nicotine (NIC) on social behaviors in the stressed model mice, and the expression of nicotinic acetylcholine receptor (nAChR) subunits or their intracellular signal-related molecules.
[Methods] The adult male C57BL/6J mice were exposed to forced swimming stress for 15 min. They were treated with NIC (0.3 mg/kg s.c.) for 7 days from the next day of exposure to stress.
[Results] The stressed mice showed the impairment of social behaviors in the social interaction test and the decreased expression of phosphorylated Akt protein in the hippocampus (HIP). These abnormalities were attenuated by repeated treatment with NIC. The expression of α7 and β2 nAChR subunit proteins was decreased in the HIP of the stressed mice. α7, but not α4β2 nAChR antagonist blocked the ameliorating effect of NIC on behavioral impairment in the stressed mice.
[Conclusions] These results suggest that repeated treatment with NIC is useful and effectively for stress-induced depressive-like behaviors. The remission of social behavior impairment by NIC may be mediated via regulating nAChR/Akt signaling in stressed mice.

1-SS-23

δ-opioid receptor inverse agonist SYK-623 reversed chronic stress-induced leaning impairment in mice

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We previously found that acute treatment of SYK-623, a new inverse agonist of δ-opioid receptors, improved restraint stress-induced learning dysfunction. In the present study, we investigated the chronic effects of SYK-623 in the chronic stress model mice. ddY mice (6-week) were administered adrenocorticotrophic hormone (once a day) and exposed to chronic mild stress for 3 weeks (herein after referred to as ACMS). SYK-623 or imipramine was administered once a day before the ACMS exposure. Short-term memory was evaluated by Y-maze. Hippocampi, adrenal glands and thymus were isolated after the behavioral test. Astrocytes and immature neurons were detected by the immunofluorescent staining in the hippocampus. ACMS induced short-term memory impairment, adrenal hypertrophy, thymic atrophy, and decreases in the density of astrocytes and immature neurons of the hippocampus. SYK-623 reversed the short-term memory impairment and the density of immature neurons, but not the others. Imipramine treatment had no effects on these symptoms. Together, chronic SYK-623 treatment reversed chronic-stress induced short-term memory impairment in imipramine resistant mice, and the increased neurogenesis may be important in this effect.

1-SS-24

Characterization of stress responsive neurons by single-cell RNA-seq

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In order to address the mechanisms of stress response of the brain, it is important to characterize each stress responsive neuron. Recently, we performed whole brain imaging at single cell resolution in Arc-dVenus reporter mice, which express destabilized Venus in activated neurons. In these mice exposed to a stressor, dVenus signals were observed in a small brain region that strongly contributes to classifying stress and control brains. Here, we examined gene expression profiles of excitatory neurons in this region and compared stress responsive neurons with their neighboring non-responsive neurons using single-cell RNA sequencing. In Arc-dVenus mice, excitatory neurons were labeled by red fluorescent protein tdTomato using the AAV-PHP.eB vector. After the mice were subjected to a single social defeat stress, tdTomato-positive and dVenus-positive neurons were individually picked up and their gene expression profiles were compared. We identified the genes of which expression are induced by the stress and those that potentially classify the stress-responsive neurons from the non-responsive neurons. This study contributes to the understanding of the molecular basis of stress response and may open the door for specific analysis of stress responsive neurons.

1-SS-25

Effect of methadone analgesia in morphine-insensitive chronic pain

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We developed the fibromyalgia-like chronic pain model in mice by using intermittent cold stress. This model mouse was found to have similarity to fibromyalgia patients in terms of pathophysiology and pharmacotherapy, which includes the loss of sensitivity to morphine. Based on the speculation that excess amounts of endogenous opioids induced by repeated stress may cause a type of opioid analgesic tolerance, we attempted to use anti-opioid NMDA receptor (NR2A subunit)-deficient mice to see the recovery of morphine analgesia. ICS-treated NR2A-KO mice successfully demonstrated the complete recovery of morphine analgesia, when given intracerebroventricularly (i.c.v.), without any change in the basal nociception threshold. The i.c.v. pre-administration of siRNA for NR2A or NR2A antagonist, (R)-CPP recovered the potency of morphine analgesia under the established pain state. Similar results were also observed with systemic (i.p.) dextromethorphan, which has NMDA receptor antagonist activity. Lastly, we found that the single administration of methadone, which has opioid agonist activity and NMDA receptor antagonist activity, showed potent analgesic actions, as seen in the case with naïve mice.

1-SS-26
Involvement of orexin-A in the regulation of central post-stroke

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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 central administration of orexin-A reduces nociceptive responses in inflammatory pain model mouse. In this study, we tested the effect of orexin-A on CPSP model mouse. Male ddY mice (5 weeks old) were subjected to 30 min of bilateral carotid artery occlusion (BCAO). Mechanical allodynia was measured by von Frey filament test. The withdrawal responses to mechanical stimuli were significantly increased on day 3 after BCAO. On day 3 after BCAO, prepro-orexin (orexin precursor) was decreased as compared with sham. The BCAO-induced mechanical allodynia suppressed by the intracerebroventricular (i.c.v.) injection of orexin-A. These effects of orexin-A were inhibited by the intrathecal injection of yohimbine (an adrenaline α_2 receptor antagonist) or WAY100635 (a serotonin 5-HT_{1A} receptor antagonist). A c-Fos (a neuronal activation marker) expression was observed in the rostral ventromedial medulla or locus coeruleus after i.c.v. injection of orexin-A. These results suggested that orexin-A plays an important role in the regulation of CPSP mediated by the descending pain control system.

1-SS-27
The allodynia induced by intrathecal injection of sulfatides.

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Glycosphingolipids (GSLs) play many important roles in cellular interaction, proliferation, vesicular transport and intracellular signal transduction. Our previous study revealed that the gene expressions of six glycosyltransferases in the GSLs biosynthesis pathway were altered in the spinal cord and dorsal root ganglion one day or 15 days after inflammation caused by complete Freund's adjuvant (CFA). We focused on CFA-induced upregulation of gal3st1 glycosyltransferase that catalyzes the synthesis of sulfatides, the sulfated GSLs in myelin structures, because the roles of sulfatides in nociceptive behavior are unclear. In this study, we intrathecally injected sulfatides into naïve mice and measured mechanical thresholds using von Frey test. Indeed, sulfatides induced mechanical allodynia within 40 min.

The report that sulfatides induce cytokine production from glial cells (Jeon SB et al., 2008 J. Immunology) leads to the hypothesis of the involvement of glial activation by spinal sulfatide during allodynia. Future studies are needed to reveal the molecular mechanisms of how sulfatides are involved in the pain transduction.

1-SS-28

DHA ameliorates repeated stress induced-chronic pain via GPR40/FFAR1

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Aims; We previously reported that the duration of postsurgical pain was prolonged by the exposure of repeated-social defeat stress (RSDS), and the prolongation was ameliorated by docosahexaenoic acid (DHA). G-protein coupled receptor 40/free fatty acid receptor 1 (GPR40/FFAR1), which is activated by long chain fatty acids, has beneficial effect to pain and emotion. In this study, we examined the involvement of DHA-GPR40/FFAR1 signaling in RSDS induced-chronic pain.

Methods; GPR40/FFAR1 wild type ($GPR40^{wt}$) and knockout ($GPR40^{ko}$) mice (9 weeks old) were exposed RSDS. Postsurgical pain was induced by plantar incision. Vehicle or DHA (25 mmol/kg) was orally administrated once a day during RSDS. Paw withdrawal thresholds (PWT) were evaluated using by von Frey filaments.

Results; In RSDS-$GPR40^{wt}$, vehicle or DHA treated mice showed decrease of PWT on day 1 after paw surgery. However, on day 7 after paw surgery, decrease of PWT disappeared in DHA treated mice but not vehicle treated mice. In RSDS-$GPR40^{ko}$, decrease of PWT occurred on day 1 after paw surgery. DHA did not ameliorate decrease of PWT in RSDS-$GPR40^{ko}$ on day 7 after paw surgery.

Conclusion; DHA-GPR40/FFAR1 signaling may be therapeutic target of chronic pain associated with emotional disorders.

1-SS-29

How much balance between excitatory and inhibitory neurons is suitable for detection of seizure liability in hiPSC-derived neurons ?

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Multi-electrode array (MEA) assays using human induced pluripotent stem cell (hiPSC)-derived neurons are expected to predict the convulsion toxicity of new drugs. Although the balance between excitatory and inhibitory inputs is important in convulsive seizure, the optimal proportion is not known. In this study, we evaluated the spontaneous firing properties and the responses to convulsants in hiPSC-derived cortical neuronal network, in which the ratio of Glutamatergic (Glu) and GABAergic (GABA) neurons are 88 : 12, 84 : 16, 74 : 26, 58 : 42, and 48 : 52. The network with a high percentage of excitatory neurons showed short synchronized burst firings (SBFs) in spontaneous firings. On the other hand, the network with high inhibitory neurons showed the SBF with long period. In drug-induced seizure activities, there was no remarkable dose responses in high percentage of excitatory neurons. On the other hand, the network with high inhibitory neurons showed significant activity changes with a lot of convulsants regardless of GABA receptor inhibitor. These results suggest that a higher proportion of GABA neurons compared with real brain is more effective in detecting drug-induced seizure toxicity.

1-SS-30

Principal component analysis for predicting the seizure liability and the mechanism of action of drugs in human iPSC-derived neural network : HESI pilot study

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Human iPSC-derived neurons are expected to be applied to toxicity evaluations in nonclinical studies. Microelectrode array (MEA), measurement system of the electrophysiological activity, are suitable to evaluate the seizure liability of drugs. We have previously reported the electrophysiological responses to several convulsive compounds using MEA in cultured hiPSC-derived neurons. However, the identification of analytical parameters to detecting seizure liability remains an important issue. We identify the analytical parameters enabling the separation of drug-induced responses between convulsants and negative control drugs, and the separation among the mechanism of action (MoA) in convulsants by using principal component analysis. Furthermore, we have succeeded in the prediction of MoA in PTZ and Linopirdine from MEA data using the identified analytical parameters. These our analysis method will be effective for detecting seizure liability and predicting the MoA of new drugs.

1-SS-31

The effect of neonicotinoids on methylation of brain genomic DNA

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The effect of neonicotinoids on methylation of brain genomic DNA

Neonicotinoids, including dinotefuran and imidacloprid, are agricultural chemicals widely used throughout the world, acting selectively on insect acetylcholine receptors. However, in recent years, it has been shown the possibility that pups' anxiety behaviors may be caused by oral administration of neonicotinoids to pregnant mice, so the influence of neonicotinoids on brain development has been concerned.

The previous studies have suggested that oral administration of neonicotinoids to the mice affects the methylation of genomic DNA, which is a regulatory mechanism of epigenetic gene expression. However, we have not identified the target genes of methylation yet. In this study, we orally administered imidacloprid and dinotefuran to ICR female mice of 12 week-old and investigated the gene methylation in cerebral cortex 4 h after administration of neonicotinoides. Then, we found increased or decreased methylation of several genes and confirmed that the methylation status of those genes was different in each drug.

In this presentation, we show the results of gene methylation and the expression profile of several genes, including *cux1* and *begain*, which play important roles in brain development, by using quantitative RCP.

1-SS-32

Antidepressant effects of XJ-Et-8 in mice chronically exposed to corticosterone

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High cortisol level in serum is one of the clinical features in depression. Exogenous administration of corticosterone (CORT) in rodents has been used as animal model of depression. Red resin of *Dracaena cochinchinensis S.C. Chen*, known as Chinese dragon's blood, has been used as a famous and precious traditional medicine since ancient times by many cultures. XJ-Et-8 is a compound extracted from Chinese dragon's blood. It has favorable effects on mouse models of Alzheimer' Diseases through the up regulating the BDNF level in the brain. The present study aimed to evaluate the XJ-Et-8 as antidepressant using a mouse model of CORT administration. CORT (20 mg/kg/day) was administered subcutaneously for 3 weeks, and XJ-Et-8 was given orally during the last 2 weeks. After corticosterone administration, mice were sequentially subjected behavioral tests: open field test, social interaction test, novelty suppressed feeding test, and forced swimming test. Corticosterone administration induced depressive and anxious behaviors and decrease of phosphorylation in AKT/mTOR/CREB signaling pathway and of BDNF contents in the prefrontal cortex. XJ-Et-8 reversed these behavioral changes, increased phosphorylation level in AKT/mTOR/CREB pathway and BDNF expression. These results suggest that the XJ-Et-8 could be a potential compound as an antidepressant via activating the AKT/mTOR/CREB pathway and BDNF expression.

1-SS-33

Sansoninto attenuates vasopressin expression and regulates 5-HTergic and DAergic systems in social isolation-reared mice.

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Sansoninto (SAT) is prescribed for annoyance and insomnia. We previously demonstrated SAT attenuates aggressive behavior in social isolation-reared (SI) mice. However the mechanism by which SAT attenuates aggression is still unknown. Arginine vasopressin (AVP) plays a critical role in the regulation of aggression in mammals. AVP is regulated by 5-HTergic and DAergic systems. In this study, the effect of SAT on expression of 5-HTergic and DAergic system-related genes and AVP was examined using SI mice.

SAT was treated for 2 weeks. Group-reared (GR) mice were used as control. AVP level in bed nucleus of the stria terminalis was measured by EIA. Expression of 5-HTergic and DAergic system-related genes were analyzed by qPCR.

SI mice showed higher AVP level than GR mice. SAT-treated SI mice showed lower AVP level as much as GR mice. Catechol-O-methyltransferase (COMT) and 5-HT_{3a} receptor mRNA level was higher in hypothalamus and amygdala of SI mice compared with GR mice. SAT-treated SI mice showed lower level of COMT and 5-HT_{3a} receptor mRNA in hypothalamus, not in amygdala, than SI mice.

These results suggest that SAT reduces the increased AVP level via regulation of hypothalamic 5-HTergic and DAergic systems.

1-SS-34
The Role of GPR4 in Myocardial Infarction

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Myocardial infarction (MI) is an ischemic heart disease caused by occlusion of coronary artery. Previous studies have shown that pH in the hearts decreases to 5.5-6.5 after MI. However, physiological significance of pH reduction remains largely unknown. Therefore, we have studied proton-sensing G protein-coupled receptors (proton-sensing GPCRs) which are activated under low pH condition. So far, four proton-sensing GPCRs have been reported. Among them, GPR4 has the highest expression level in mouse heart. Thus, we studied the role of GPR4 in pathological responses after MI, to elucidate the physiological significance of pH reduction.

We found that GPR4 is highly expressed in vascular endothelial cells which express cell adhesion molecules and promote infiltration of leukocytes. Expression level of cell adhesion molecules was significantly suppressed in GPR4 KO mice. Infiltration of neutrophils and expression of inflammatory cytokines were also suppressed. Cardiac function after MI was improved in GPR4 KO mice compared to WT mice.

In this study, we demonstrated that pH decrease after MI activates GPR4 and induces inflammatory responses leading to heart dysfunction. These results indicate that GPR4 can be a new therapeutic target for the treatment of MI.

1-SS-35

Eccentric regulation of P2Y6 receptor signaling in pressure overlaod-induced cardiac hypertrophy

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Purinergic P2Y6 receptor (P2Y6R) is a member of G protein-coupled receptor (GPCR) activated by uridine nucleotides. We previously reported that MRS2578, a specific inhibitor of P2Y6R, suppressed cardiac remodeling and dysfunction induced by pressure overload (**EMBO J**, 2008). We thus hypothesized that P2Y6R deficient mice would show the same phenotype and performed transverse aortic constriction (TAC) to induce cardiac pressure overload in P2Y6R deficient mice. Contrary to our expectation, P2Y6R deficiency exacerbated cardiac remodeling induced by pressure overload. Surprisingly, transgenic mice with cardiomyocytes-specific overexpression of P2Y6R was also found to worsen cardiac remodeling after pressure overload. We thus speculated that MRS2578 could activate P2Y6R-dependent cardioprotective signaling pathway. We found that MRS2578 upregulated survival factors in mouse hearts, such as SOD2 and SIRT3 in a P2Y6R-dependent manner. We further found that MRS2578 treatment multimerized P2Y6R proteins, which were translocated from plasma membrane to cytosol. These results suggest that MRS2578-induced changes in localization and protein quality of P2Y6R is essential for the activation of cardioprotective signaling pathways.

1-SS-36
TRPC3-Nox2 axis mediates ATP-induced cardiomyocyte atrophy

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The heart is capable of adapting to various environmental stresses by flexibly changing its structure. Hemodynamic overload (or mechanical stress) and cardiotoxic drugs, such as doxorubicin, cause pathological cardiac remodeling in which abnormal production of reactive oxygen species (ROS) plays a critical role. We have recently showed that functional coupling between transient receptor potential canonical (TRPC) 3 and NADPH oxidase 2 (Nox2) mediated aberrant ROS production during pathological remodeling of mouse hearts. In this study, we found that extracellular ATP caused atrophy of neonatal rat cardiomyocytes (NRCMs), which was attenuated by silencing TRPC3 or Nox2 genes. ATP increased expression of muscle atrophy-related E3 ubiquitin ligase, MAFbx. TRPC3 and Nox2 were responsible for ATP-induced ROS production and atrophy of NRCMs. We demonstrated that ATP treatment caused physical interaction between TRPC3 with Nox2 in TRPC3 and Nox2-overexpressing HEK293 cells. The ATP-induced increase of TRPC3-Nox2 interaction was also observed in NRCMs as well. In summary, ATP may induce cardiomyocyte atrophy through increases in MAFbx expression and ROS production by activating TRPC3-Nox2 functional coupling.

1-SS-37

The effect of anti-adiponectin antibody on experimental macular edema

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The macular edema is caused with the increase of vascular endothelial growth factor (VEGF) in diabetic macular edema (DME) and retinal vein occlusion (RVO) patients. However, there are some drawbacks after anti-VEGF therapy. In an earlier report, adiponectin which is an adipokine secreted from adipocytes was increased in the retina of streptozotocin-induced diabetic retinopathy murine model. The purpose of this study was to investigate the involvement of adiponectin in the pathophysiology of retinal vascular hyperpermeability.

We investigated in VEGF and adiponectin levels in vitreous humor of DME and proliferative diabetic retinopathy (PDR) patients by ELISA. Moreover, we performed adiponectin level in murine RVO model by RT-PCR and Western blotting. To evaluate the effect of anti-adiponectin antibody, retinal thickness was measured by HE staining.

The adiponectin level with the vitreous body of DME patients was higher than PDR patients. Interestingly, both mRNA and protein of adiponectin were increased in the retina of murine RVO model, and the increase of the retinal thickness was ameliorated by anti-adiponectin antibody therapy. These data indicate that adiponectin may represent one of potential therapeutic targets for retinal edema in DME and RVO patients.

1-SS-38

A Protective role of NOX1/NADPH oxidase against cytotoxic components of cigarette smoke extracts in cardiomyocyte

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A superoxide-generating NADPH oxidase (NOX) has been reported to mediate cytotoxicity induced by cigarette smoke components. However, the underlying molecular mechanisms and NOX isoform involved have not been fully clarified. Among NOX isoforms identified so far, NOX1 and NOX4 were expressed in rat H9c2 cardiomyocytes. When H9c2 cells were exposed to acrolein or methyl vinyl ketone (MVK), major toxic components of cigarette smoke extracts, a dose-dependent decline in cell viability was observed. Unexpectedly, disruption of *Nox1* significantly exacerbated cytotoxicity induced by acrolein or MVK. The levels of total and reduced glutathione (GSH) were significantly reduced in *Nox1*-disrupted H9c2 clones. In these clones, expression of multidrug resistance-associated protein 1 (MRP1), which mediates glutathione efflux, was significantly up-regulated. Treatment of reversan, an MRP1 inhibitor, partially but significantly blunted the cytotoxicity of acrolein and MVK in *Nox1*-disrupted cells. Taken together, NOX1/NADPH oxidase regulates the expression of MRP1 to maintain intracellular GSH levels in cardiomyocytes and protect against cytotoxic components of cigarette smoke extracts.

1-SS-39

A systems pharmacology approach to the search for novel myelination drugs

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Oligodendrocytes are responsible for myelin sheath formation in axons and are compromised in demyelinating diseases, but no treatment has been established to promote their regeneration. In this study, we utilized next-generation sequencing data to analyze the effect of miconazole and clobetasol —two inducers of myelination—on the transcriptome in mice. We found that sterol regulatory element binding factor (SREBF) was involved in myelination. We then screened for SREBF-activating compounds *in silico* and found the PPARα agonist fenofibrate. Subsequently, we created a transgenic zebrafish line that expresses mCitrine fluorescent protein in oligodendrocytes, under myelin basic protein promotor control. We administered fenofibrate to this zebrafish and performed *in vivo* imaging by high content screening, and found increased fluorescence signal in oligodendrocytes. Moreover, administering gemfibrozil, another PPARα agonist, to the zebrafish also resulted in a similar increase. This increase induced by fenofibrate or gemfibrozil was suppressed by simultaneous administration of the SREBF inhibitor fatostatin, suggesting that fenofibrate and gemfibrozil induced myelination via SREBF. This approach enables a high-throughput search for novel myelination drugs.

1-SS-40

Effect of a Nurr1 ligand amodiaquine on pathology of intracerebral hemorrhage in mice

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Effect of a Nurr1 ligand amodiaquine on pathology of intracerebral hemorrhage in mice
Intracerebral hemorrhage (ICH) is characterized by high mortality and neurological deficits caused by the formation of hematoma in the brain parenchyma. Nurr1 is an orphan nuclear receptor involved in the suppression of pro-inflammatory responses of microglia and astrocytes as well as the maintenance of survival of midbrain dopaminergic neurons. Here we addressed whether Nurr1 serves as a target for ICH therapy, using an anti-malarial drug amodiaquine (AQ) that has been reported to possess agonistic activity on Nurr1. ICH was induced in the striatum of male ICR mice by injection of type VII collagenase. AQ (40 mg/kg) was administered intraperitoneally at 3 h after ICH, and thereafter, every 24 h. Nurr1 expression was observed in microglia in the peri-hematoma region, but not in the contralateral hemisphere. ICH was accompanied by activation of microglia/macrophages and astrocytes in the peri-hematoma region, and also by increased expression of mRNAs encoding inflammatory mediators such as IL-1band CXCL2. These inflammatory responses were markedly attenuated by AQ. Moreover, AQ improved motor function of mice after ICH. These results indicate that Nurr1 activation alleviates pathogenic events associated with ICH.

1-SS-41

Resveratrol attenuates microglial inflammation induced by oxygen glucose deprivation via blocking hmgb1 damp signaling

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OBJECTIVE: To investigate whether the HMGB1 DAMP signaling pathway is involved in resveratrol anti-oxygen glucose deprivation (OGD)-induced microglial inflammatory processes and explore its underlying mechanisms.
METHODS: Cell viability was tested by MTT assay to determine the appropriate resveratrol and EX527 concentration and OGD time, and the cells were divided into four groups: Control, OGD +DMSO, OGD+RES and OGD+RES+EX527, ELISA. Rt-PCR and western blot were used to detect inflammatory factors and HMGB1 signaling pathway-related protein changes. WB and immunofluorescence were used to demonstrate the localization of HMGB1 in cells, the acetylation level of HMGB1 and the interaction between HMGB1 and Sirt1 were detected by CoIP. Different groups BV2 cells were co-cultured with primary mouse neurons, and the release of HMGB1 was observed and the level of LDH in the supernatant was detected.
RESULTS: We determined that RES (100umol), EX527 (100umol) and OGD3h were the optimal treatment conditions. RES inhibited the increase of inflammatory mediators and HMGB1 signaling pathway-related proteins and reduce the increase of HMGB1 level in cell supernatant after OGD, and EX-527 reversed this effect; immunofluorescence indicated that RES can limit HMGB1 in cells, however, different from the change of HMGB1 in the culture medium, there was no significant difference in the mRNA level of HMGB1 in each group, suggesting that the increase of HMGB1 level in supernatant after hypoxia is mainly due to the increase of active secretion rather than synthesis. CoIP results showed that the binding of HMGB1 to deacetylase SIRT1 was decreased and the level of acetylation was decreased after OGD. RES could increase the interaction between HMGB1 and sirt1 and increase the acetylation level of HMGB1 but EX527 reduced this interaction.

1-SS-42

Role of microglia in Alexander disease: the influence of microglia depletion

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Alexander Disease (AxD) is a rare neurodegenerative disease which is caused by dominant gain of function mutations in the GFAP gene. In AxD astrocytes show reactive phenotype with Rosenthal fibers which comprise of GFAP accumulations with aB-crystalline. Although AxD is thought to be an astrogliopathy, emerging evidence indicates that the inflammatory response including the microglial activation is induced in AxD. Accordingly, we previously observed the increase in the number of Iba1-positive microglia in the hippocampus from hemizygotes of 60TM AxD model mice which express human GFAP with R239H mutation (60TM mice). To understand the role of microglia in AxD, we depleted microglia from 60TM mice by treatment with PLX5622 (PLX), a CSF-1 receptor antagonist, between P21 and P42. PLX eliminated almost all Iba1-positive signals and its mRNA level in the hippocampus. Surprisingly, PLX increased GFAP staining and Fluoro-Jade B staining, suggesting exacerbation of AxD pathogenesis. PLX also augmented mRNA levels of reactive astrocyte markers such as *Vim*, *C3* and *Cd44*. These data suggest that microglia may play a beneficial role in AxD pathogenesis by restraining the astrocyte to show reactive phenotype in this AxD model at early-developmental stage.

1-SS-43

Changes of progranulin in activated microglia after cerebral ischemia

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Progranulin (PGRN), a cysteine-rich secretory protein, is implicated in neuronal protection. In the central nervous system, it has been reported that PGRN protects against neuroinflammation after cerebral ischemia. On the other hand, granulin (GRN), which is cleaved from PGRN by neutrophil elastase, exerts pro-inflammatory effects. However, roles of PGRN and GRN after cerebral ischemia have not been fully determined. In this study, we examined time-course of changes in the levels of PGRN and GRN and their cellular sources after cerebral ischemia. A rat microsphere-embolism (ME) model and primary cultured microglia isolated from cerebral cortex were used. Protein and mRNA levels of PGRN were increased in the ischemic region of cerebral cortex on day 3 after ME. Furthermore, expression of PGRN was increased in activated microglia after ME. Elastase activity in cerebral cortex was increased on day 1 after ME. GRN protein was increased on day 1 after ME. These results suggest that increased elastase activity causes cleavage of PGRN, and then GRN may promote inflammatory responses after ischemia. Thus, the changes in the levels of PGRN and GRN might contribute to pathological alterations in ischemic disorders.

1-SS-44

Intra-hippocampal injection with mouse bone marrow-derived microglia-like cells contribute to amyloid-β clearance and improvement of memorial dysfunction in a mouse model of Alzheimer's disease.

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Accumulation of amyloid-β peptides (Aβ) in the brain triggers the onset of Alzheimer's disease (AD). Therefore, promotion of Aβ clearance is a promising strategy for AD therapy. We previously demonstrated that primary-cultured rat microglia phagocytose Aβ, and the transplantation with rat microglia ameliorates the Aβ burden in brains of Aβ-injected rats. In this study, we demonstrated that stimulation with colony-stimulating factor-1 (CSF-1) efficiently differentiated mouse bone marrow (BM) cells into BM-derived microglia-like (BMDML) cells. BMDML cells effectively phagocytosed Aβ in vitro, and it was comparable to that of primary-cultured mouse microglia. We further found that intra-hippocampally injected BMDML cells migrated directionally toward Aβ plaques in a mouse model of AD in comparisons with a simulation assuming a uniform distribution of cells. Finally, we detected Aβ phagocytosis by BMDML cells concomitant with a reduction in the number and area of Aβ plaques and amelioration of the cognitive impairment in the mouse model. Our results suggest that BMDML cells could be used in cell-based disease-modifying therapies against AD.

1-SS-45

Anti-tumor effects of astaxanthin and adonixanthin on glioblastoma cells

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Glioblastoma (GBM) is one of the most lethal brain tumor arised from glial cells. The chemotherapy is important to improve the prognosis of GBM. Although temozolomide has been used as a first line drug for GBM, some patients could not prolong their survivals. Thus, it is required to develop new drugs which have effectiveness on GBM. Astaxanthin was reported to have anti-tumor effects on lung cancer, liver cancer and so on. This study was performed to clarify whether both astaxanthin and its intermediate compound adonixanthin have anti-tumor effects on GBM cells.

We evaluated the ability of cell proliferation and migration by WST-8 and scratch assay. Moreover, we evaluated the expression of some proteins which were related to tumor progression by immunoblot. Furthermore, we performed ROS assay of astaxanthin and adonixanthin. Astaxanthin and adonixanthin inhibited the cell proliferation and migration of GBM cells. Moreover, the phosphorylation of ERK and Akt were decreased. The suppression of ROS by astaxanthin and adonixanthin may be related to the reductions of phospho-ERK and Akt.

In conclusion, these results indicate that astaxanthin and adonixanthin inhibit the cell proliferation and migration of GBM cells via suppression of ERK and Akt signaling pathways.

1-SS-46

Regulation by Smurf2^{Thr249} phosphorylation of stemness of glioma-initiating cells.

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We have recently demonstrated that phosphorylation of SMAD specific E3 ubiquitin protein ligase 2 (Smurf2) at Thr249 (Smurf2^{Thr249}) plays an essential role for maintenance of stemness of the mesenchymal stem cells (Iezaki, Hiraiwa et al., *Development* 2018). Glioblastoma (GBM) is the most common high-grade malignant glioma in adults. Emerging evidence indicates that glioma-initiating cells (GICs) contribute to drug resistance and tumor recurrence owing to their ability for self-renewal. Here, we show that phosphorylation of Smurf2^{Thr249} plays an important role for maintenance of stemness of GICs and GBM progression. Smurf2^{Thr249} phosphorylation was markedly decreased in GBM patients as well as in GBM model mice. Infection of Smurf2(T249A) mutant, in which the threonine was replaced with an alanine to prevent phosphorylation, significantly increased not only sphere formation ability of human GICs but tumor progression in a GBM mouse model. On the contrary, Smurf2 phospho-mimetic mutant (Smurf2(T249E)) decreased both of them. These findings highlight a critical role of Smurf2^{Thr249} phosphorylation in maintenance of stemness of GICs and GBM tumorigenesis, thereby providing a novel target in GBM.

1-SS-47

mTORC1 in osteoblastic niche regulates progression of acute myeloid leukemia.

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Hematopoieticstem cells(HSCs) and leukemic stem cells(LSCs) have self-renewal ability to maintain normal hematopoiesis and leukemia propagation, respectively. Recently, it has been reported that bone forming osteoblasts provide a microenvironment for LSCs and are implicated in pathogenesis and progression of leukemia as an osteoblastic niche in bone marrow. The mTOR complex 1 (mTORC1), a member of the serine/threonine kinases, is known to regulate the cellular function in various cell types. Although the role of osteoblastic mTORC1 on bone mass accrual has been investigated, here we show a critical role of mTORC1 in regulating normal hematopoiesis and leukemia progression through its expression in osteoblasts in mice. Using a mouse models of acute myeloid leukemia (AML), we revealed that AML cells enhance the mTORC1 activity in osteoblasts *in vivo* and *in vitro*. Subsequent analyses determined that inactivation of *Tsc1*, a negative regulator of mTORC1, in osteoblasts results in a marked acceleration of AML. These findings highlight a critical role of mTORC1 in normal hematopoiesis and leukemia propagation, at least in part, through its expression in osteoblastic niche.

1-SS-48

Identification of an ubiquitin ligase that stabilizes Smad6 and inhibits BMP signaling

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Smad molecules (Smad1-8) mediate BMP/TGF-β signaling and play important roles in various biological responses such as development and cancer progression. However, the mechanisms regulating stabilities of Smad proteins remain largely unknown. We found that Smad6 protein is more stable than other Smad family proteins, and revealed the molecular mechanisms that regulate Smad6 protein stability.

We first searched for the Smad6-interacting proteins that can affect Smad6 protein stability. The LC-MS/MS analysis revealed an ubiquitin ligase that directly interacts with Smad6. We found that the ubiquitin ligase polyubiquitinated Smad6, but unexpectedly promoted Smad6 protein stability. The ubiquitin assay using various Smad6 mutants identified the 7 lysine residues of Smad6 that are ubiquitinated by the ubiquitin ligase. Consistently, a Smad6 mutant in which the 7 lysine residues were replaced by arginine (Smad6 KR) did not undergo polyubiquitination by the ubiquitin ligase, and the protein stability of Smad6 KR was much lower than that of Smad6 WT. Smad6 was reported to worsen clinical condition of breast cancer by inhibiting BMP signaling. Interestingly, the tumorigenicity of Smad6 KR was much lower than that of Smad6 WT.

1-SS-49

Anti-tumor effects of L-type amino acid transporter 1 (LAT1) inhibitor in a syngeneic and orthotropic mouse model for melanoma

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Melanoma is the most malignant form of skin cancer, which originates from the pigment-producing melanocytes in the basal layer of epidermis. L-type amino acid transporter 1 (LAT1) is an amino acid transporter that transports most of the essential amino acids. LAT1 is upregulated in various cancers, including melanoma, and contributes to supporting cancer cell proliferation. Therefore, LAT1 is regarded as a promising molecular target of novel anti-cancer therapeutics. In this study, we established a syngeneic and orthotropic mouse model for melanoma to examine the anti-tumor effects of LAT1 inhibitor. B16F10 mouse melanoma cells were orthotopically injected into footpads of C57BL/6J mice. One week after the inoculation, intravenous injection of LAT1 inhibitor was started and continued once-daily for two weeks. The tumor size on footpads of LAT1 inhibitor-treated mice was significantly reduced compared to those of saline-treated control mice, demonstrating the anti-tumor effects of LAT1 inhibitor. Our syngeneic and orthotropic model will be useful to evaluate the therapeutic efficacy of LAT1 inhibitors for melanoma in the presence of intact immune system, and also to investigate its detailed mechanisms of action as anti-tumor therapeutics.

1-SS-50

Suppression of colorectal cancer cell growth by combinational inhibition of amino acids transporters

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Alanine-serine-cysteine transporter 2 (ASCT2), a neutral amino acids transporter, plays a central role in sustaining glutamine metabolism. L-type amino acid transporter 1 (LAT1), a large neutral amino acids transporter, transports most of the essential amino acids. Both of them are highly expressed in various type of cancers. In the present study, we investigated the combinational effect of ASCT2 inhibitor and LAT1 inhibitor in four colorectal cancer cell lines in cell proliferation experiments *in vitro*. Treatment with ASCT2 or LAT1 inhibitor alone showed limited growth inhibition in all the tested cell lines. Treatment with the combination of two inhibitors showed prominent synergistic growth inhibition in two cell lines, additive growth inhibition in one cell line, while there was no obvious combinational effect in the remaining one cell line. LAT1 and ASCT2 expression, transport function, amino acids content and signaling in these cell lines will be further studied, to reveal the cellular determinants underling the difference among cell lines. The results of this study may contribute to propose combinational treatment for colorectal cancers using inhibitors of the two amino acid transporters.

1-SS-51

A pilot study on the role of tissue kallikrein in the nafamostat-induced hyperkalemia.

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Nafamostat has been reported to cause the drug-induced hyperkalemia. Nafamostat has an inhibitory effect on the serine proteases including tissue kallikrein. The study aimed to examine involvement of tissue kallikrein in the nafamostat-induced hyperkalemia.

Seven-week-old male Wistar-Imamichi rats were used. Nafamostat (6～18mg/kg) or amiloride (3mg/kg), a potassium-sparing diuretic and a positive control, was i.p. administered. Urine and blood were collected 6 h after administration. Potassium and creatinine (Cr) were measured by the ion-electrode and Jaffe methods, respectively. Tissue kallikrein was measured using the synthetic peptide of the substrate.

In the nafamostat group (n=5), serum potassium and urinary potassium and tissue kallikrein were 5～5.3 mmol/L (control group 4.6±0.2, mean±SD, n=4), 0.33～0.58 mmol/mg Cr (0.56±0.14) and 0.02～0.10μmol/mg Cr (0.11±0.04). In the amiloride group (n=3), they were 6.3±0.6 mmol/L (4.7, mean, n=2), 0.28±0.20 mmol/mg Cr (1.02), 0.06±0.01μmol/mg Cr (0.26), respectively. It was suggested that serum potassium increased and urinary potassium and kallikrein decreased in both groups. In the nafamostat group, redness on the peritonea and hemorrhagic ascites were observed.

Nafamostat-induced hyperkalemia may be involved in the sodium channel inhibition. Intraperitoneal findings may be caused by the anticoagulant effect. Further studies are necessary on the i.v. administration of nafamostat.

1-SS-52

The effect of oral administration of cloperastine on micturition reflex in mice

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We have previously reported that cloperastine (CP), a centrally acting non-narcotic antitussive, improved micturition dysfunctions in rodents. Although clinical application of CP is expected, it is unknown whether oral administration of CP affects micturition reflex. In this study, we measured micturition functions in awake and anesthetized mice chronic orally administered with CP. **Method** Female BALB/c mice were purchased. CP 20mg/kg was orally administered once a day for 14 days and used for the following two experiments. 1) Real-time micturition activity of freely moving mice was measured for 24 hours by using a sequential urine collection and recording system developed by us. 2) The intravesical pressure of the urethane-anesthetized mice was measured by conventional single cystometry. **Result** 1) In the awake mice, CP significantly increased voiding frequency, total voided volume and voiding duration in the dark period and also for 24 hours compared with the control group. 2) In the anesthetized mice, mean urine flow rate was significantly decreased, and voiding duration tended to increase in CP group compared with control. These results suggest that chronic oral administration of CP, at cough suppressant dose, may affect micturition reflex in mice.

1-SS-53

Increased oxidative stress mediated by NOX1/NADPH oxidase promotes liver injury in a mouse model of nonalcoholic fatty liver disease

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A critical role of oxidative stress in the development of nonalcoholic fatty liver disease (NAFLD) has been reported. To clarify the source of oxidative stress, hepatic expression of the superoxide-generating NADPH oxidase isoforms was examined in high-fat and high-cholesterol (HFC) diet-fed mice. The level of NOX1, but not of NOX2 or NOX4 mRNA was significantly elevated in the liver of mice fed HFC diet for 8 weeks. Increased levels of serum alanine aminotransferase and hepatic cleaved caspase-3 in HFC-fed wild-type mice (WT) were significantly ameliorated in mice deficient in *Nox1* (Nox1-KO). Formation of nitrotyrosine adducts, a marker of peroxynitrite-induced injury, was apparent in hepatic sinusoids of HFC diet-fed WT, which was significantly suppressed in Nox1-KO. In fact, NOX1 mRNA was predominantly expressed in the fraction of liver sinusoidal endothelial cells (LSECs). In primary cultured LSECs, palmitic acid, the most abundant saturated free fatty acid in plasma, dose-dependently up-regulated NOX1 mRNA. Accordingly, increased oxidative stress mediated by NOX1/NADPH oxidase in LSECs may promote liver injury through peroxinitrite formation in the development of NAFLD.

1-SS-54
Immune system observation in a murine sarcopenic model

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Muscle atrophy is a result of aging and disease, and can compromise physical function and impair vital metabolic processes. Low levels of muscular fitness, known as ‘flail', contribute greatly to weakness, disability, and immobility, and lead to increased hospitalization and loss of independence.

To investigate the correlation between muscle atrophy and aging-related immune deterioration, a dissected sciatic nerve sarcopenic mouse model was established and the fraction of immunological cells, including T cells, B cells, macrophages, natural killer cells, and neutrophils, was determined. Eight weeks after sciatic nerve dissection, the volume of both hind legs was evaluated based on acquired magnetic resonance imaging. Primary splenocytes and biceps femoris muscle-derived cells were isolated and labeled by cellular markers. The subpopulation of the cells was then detected using flow cytometry. T and B cell lineages were significantly suppressed in the sarcopenic model compared with control mice, and the fraction of macrophages and natural killer cells also tended to increase.

In conclusion, muscular loss appears to affect the immunological system by modulating humoral immunity and the cell-based immunology response. Further functional analysis of individual cell subsets could further determine the influence of sarcopenia on immune system improvement.

1-SS-55

The effects of dasatinib on corticosteroid insensitive airway inflammation induced by lipopolysaccharide

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Corticosteroid resistance is observed in some patients of COPD and severe asthma, resulting in difficult to control of airway inflammation. We previously reported that repeated dosed lipopolysaccharide (LPS) induced corticosteroid insensitive airway inflammation in mice. And recently, some groups reported that Src was important to inflammatory responses in COPD and asthma models of mice. Thus, we determined the effects of dasatinib, a src inhibitor, on repeated dosed LPS-induced airway inflammation in mice.

A/J mice were intranasally exposed to LPS twice daily for 3 days, and intranasally treated with dasatinib 2 hr before each LPS exposure. One day after the last LPS exposure, bronchoalveolar lavage fluid (BALF) was collected. The number of inflammatory cells and cytokines expression levels in BALF were measured by flow cytometry and ELISA, respectively.

LPS increased the number of total cells, macrophages, and neutrophils, and CXCL1 and TNF-α levels. Dasatinib significantly reduced BALF cells and the CXCL1 level. Dasatinib also reduce the TNF-α level. These results suggested that src was involved in airway inflammation and dasatinib will provide a new therapeutic agent for corticosteroid insensitive airway inflammation.

1-SS-56
Deep learning estimates neuronal depolarization amplitudes

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Patch-clamp recordings are useful to investigate the electrophysiological dynamics of neurons at single synapse level with high time resolution. However, subthreshold membrane potentials (Vm) recorded from *in-vivo* animals include complex synaptic inputs from thousands of presynaptic neurons, and it is technically difficult to remove artifacts induced by respiration and blood vessel pulsation. The goal of this research is to quantify the amplitude of a synaptic input in *in-vivo* synaptic bombardments using deep learning, which is designed to recognize natural images with high accuracy. We recorded spontaneous Vm fluctuations from hippocampal CA1 pyramidal cells in anesthetized mice and a pseudo-ideal excitatory post synaptic potential (EPSP) from a CA1 pyramidal cell in a hippocampal slice. The *in-vivo* Vm fluctuations were randomly phase-shifted to distort the waveform of intrinsic synaptic inputs and were superimposed with a single EPSP with various amplitudes, then we yielded surrogate Vm images with and without EPSPs. We trained ResNet, a deep learning model, with this dataset to estimate the amplitudes of EPSPs embedded in Vm fluctuations. We succeeded in reducing the mean error of the prediction to a level of the standard deviation of spontaneous Vm fluctuations.

1-SS-57

Assessment of seizure liability in human iPSC-derived neurons using AI-HESI NeuTox Pilot study-

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Micro-electrode array (MEA) assay using human iPSC-derived neurons are expected to one of in vitro assays to predict the toxicity and predict the mechanism of action of drugs. MEA subteam of NeuTox Committee in Health and Environmental Science Institute (HESI) have started the pilot study for the prediction of seizure liability of drugs. In this study, we aimed to develop an analytical method enabling the evaluation of toxicity of convulsants using deep learning. Human iPSC-derived cortical neurons and astrocytes were cultured on 24-wells MEA plate for extracellular recording using MED64 Presto. HESI twelve compounds were tested at 5 concentrations for each compound (n>6). We firstly had artificial intelligence (AI) learned the data of convulsants and the data of non-convulsants. Next this AI predicted the Toxicity of the data not used for learning. The toxicity probability of unlearned sample data was 90% or more, and the toxicity probability of the unlearned convulsants was also 80% or more. In addition, the negative probability of non-convulsants was more than 80%. These results indicated that this AI analysis method is useful for predicting the convulsion toxicity using hiPSC-derived neurons.

1-SS-58

Search for preventive drugs against oxaliplatin-induced peripheral neuropathy through drug repositioning

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Background: Oxaliplatin causes oxaliplatin-induced peripheral neuropathy (OIPN). Because OIPN reduces patients' quality of life (QOL), the development of preventive agents against OIPN is an important area of research. In this study, we searched for preventive drugs against OIPN by drug repositioning, using large-scale medical data, and experimentally validated the findings with neuron-like cells and OIPN rat models.

Methods and Results: First, we searched for approved drugs that cancel the gene expression change caused by oxaliplatin, using the drug discovery tool LINCS, and found 23 approved drugs that met the requirements. Candidate drugs were then evaluated for their mitigating effects on OIPN, using the FDA Adverse Event Reporting System (FAERS) database, and Drug X was found to significantly reduce the risk of patients developing OIPN. Using PC12 cells, we observed significant improvement in oxaliplatin-associated axonal damage with Drug X treatment. In addition, in vivo experiments showed that Drug X significantly reduced the expression of oxaliplatin-induced neuropathy in OIPN rat models.

Conclusion: This study suggested that drug repositioning of Drug X could allow it to be used as a preventive agent for OIPN.

1-SS-59

Development of a radiolabeling method of affibody molecules using the cell-free translation system and F-18 labeled amino acid

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Positron Emission Tomography (PET) is a non-invasive molecular imaging technique for clinical diagnostic with the detection of pathological lesions. Small radioactive molecules such as fluorodeoxyglucose have been mainly used as PET tracers. However, they have some challenges in the aspect of their specificity. Recently, small protein ligands (6-7 kDa), affibody molecules, are attracting increasing interest as an innovative PET tracer owing to their promised high specificity and affinity. In this study, we propose a novel radiolabeling method for protein with F-18, termed TAG-encoded cell-free protein synthesis system with F-18 labeled amino acid, to use affibody molecules as PET tracers. Furthermore, we show the successful preparation of radiolabeled affibody molecules targeted to HER2 and PD-L1 with this method. A small animal PET imaging with F-18 labeled affibodies surely demonstrated the accumulation of tracers in tumors expressing HER2 or PD-L1. This technique will be a powerful tool for the evaluation of potential candidates for PET tracers in preclinical settings because of its facile radiolabeling of protein just by adding the template DNA plasmids.

1-SS-60

A rapid procedure for measurement of plasma concentration of a molecular target anti-cancer drug with diamond sensor.

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Molecular target anticancer drugs have less adverse effects than conventional compounds but often provide patients with unfavorable events. Development of an appropriate administration protocol that alleviate the adverse effects and maximize the desirable effects requires monitoring of drug concentrations in individuals. This strategy is currently inaccessible, owing to no convenient method usable at a clinical site. Furthermore, as for recent drugs whose therapeutic windows remain unevaluated, it is difficult to control the adverse effects. To address these shortcomings, we describe a rapid and simple procedure for determination of the concentrations in blood samples using lenvatinib, a multi-kinase inhibitor, as a test reagent. This method stems from electrochemical measurement with diamond sensor that induces more stable reaction than classical materials. When guinea-pig plasma mixed with lenvatinib was examined, the sensor could detect a clinically relevant concentration of ≥ 1 μM. Time necessary for all the processes including sample's pretreatment did not exceed 10 minutes. This procedure may contribute to advances in personalized medicine.

1-SS-61

Origins and targets of HMGB1 essential for bortezomib-induced peripheral neuropathy in mice: distinct profiles in the development phase and sustained period

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Given our previous evidence that MΦ-derived HMGB1 participates in paclitaxel-induced peripheral neuropathy, we studied possible role of HMGB1 in the neuropathy caused by bortezomib (BTZ), a proteasome inhibitor used for treatment of multiple myeloma. To create BTZ-induced peripheral neuropathy (BIPN) in mice, BTZ was administered i.p. 3 times weekly for 2 weeks. The development of BIPN was prevented by an anti-HMGB1-neutralizing antibody (Ab) and antagonists of RAGE or CXCR4, but not Toll-like receptor 4, among targets for HMGB1, and by minocycline, an inhibitor of MΦ/microglia, ethyl pyruvate, known to inhibit HMGB1 release from MΦ, or liposomal clodronate, a MΦ depletor. Only Ab, the RAGE antagonist or minocycline, when given once on day 14, reversed the sustained BIPN. In the dorsal root ganglion, MΦ accumulation was detected on day 3, but not 14, of BTZ treatment, and RAGE upregulation was found on day 14. BTZ directly caused HMGB1 release from MΦ-like RAW264.7 cells. Our data suggest the involvement of RAGE and CXCR4 activation by MΦ-derived HMGB1 in the development of BIPN, and of RAGE activation by HMGB1 released from non-MΦ cells in the sustained period of BIPN.

1-SS-62

The inhibitory actions of methylcobalamin on mechanical allodynia in herpes murine model

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Methylcobalamin (MeCbl) is an analog of vitamin B_{12} used to relieve peripheral neuropathy. In this study, we examined whether MeCbl inhibited mechanical allodynia (acute herpetic pain -AHP- and postherpetic neuralgia -PHN-) in mice infected with herpes simplex virus-1 (HSV-1). Mice were inoculated transdermally with HSV-1. Herpes zoster-like skin lesion peaked on day 7 after the inoculation, and was completely healed by day 20. Mechanical allodynia peaked on day 7-10 (AHP) post-inoculation, and was continuously observed after rash healing (PHN). Single administration of MeCbl inhibited mechanical allodynia in AHP phase. The anti-allodynic action of MeCbl was suppressed by naloxone, an opioid receptor antagonist, but not naloxone methiodide which has limited access to the CNS. Furthermore, repetitive administration of MeCbl from day 5 post-inoculation suppressed both AHP and PHN, and also promoted recovery of the peripheral nerve fibers decreased by HSV-1 infection in the footpad skin. These results suggest that activation of endogenous opioid system in the CNS and promotion of recovery of decreased peripheral nerve fibers are involved in the anti-allodynic actions of MeCbl in HSV-1-infected mice.

1-SS-63

Cathepsin E-dependent production of elastase in neutrophils induces mechanical allodynia in experimental autoimmune encephalomyelitis

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In multiple sclerosis (MS) patients, pain is a frequent and disabling symptom. However, the underlying mechanisms of neuropathic pain in MS patients is poorly understood. In the present study, we have demonstrated that cathepsin E (CatE) in neutrophils is required for mechanical allodynia in experimental autoimmune encephalomyelitis, an animal model of MS. We show that CatE-deficient (CatE -/-) mice were highly resistant to myelin oligodendrocyte glycoprotein (MOG 35-
55)-induced mechanical allodynia. After MOG 35-55 immunization, neutrophils immediately accumulated in the dorsal root ganglion (DRG) where neutrophils released elastase in a CatE-dependent manner. Furthermore, sivelestat, a selective neutrophil elastase inhibitor, suppressed mechanical allodynia caused by adoptively transferred MOG 35-55 -stimulated neutrophils. Neutrophil-
driven increased pain perception was mediated through the activation of protease-activated receptor 2 in DRG neurons. Activation of neutrophils by MOG 35-55 was mediated by toll-like receptor 4. Our
findings suggest the mechanism of driving mechanical allodynia caused by MOG 35-55 and new strategy for preventing pain in MS patients.

1-SS-64

Role of the cystathionine γ-lyase/H_2S pathway in paclitaxel-induced HMGB1 release from macrophages and its impact on the pathogenesis of peripheral neuropathy in mice

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We have reported that inhibitors of cystathionine γ-lyase (CSE), an H_2S-generating enzyme, reverse paclitaxel (PCT)-induced peripheral neuropathy (PIPN) in rats (Neuroscience 2011;188:148-156), and that PIPN in rats and mice involves macrophage-derived HMGB1, a DAMP molecule (Neuropharmacology 2018;141:201-213). Thus, we investigated a possible crosstalk between CSE/H_2S and HMGB1 pathways in macrophages and its implication for PIPN in mice. Repetitive i.p. administration (days 0, 2, 4 and 6) of PCT caused mechanical allodynia, as assessed by von Frey test, which was prevented by repeated i.p. administration of DL-propargylglycine (PPG), a CSE inhibitor. A single administration of PPG as well as β-cyano-L-alanine (BCA), another CSE inhibitor, reversed the established PIPN. In macrophage-like RAW264.7 cells, PCT at 1 μM produced HMGB1 release, an effect abolished by PPG or BCA. Na_2S, an H_2S donor, at 30-100 μM also caused HMGB1 release from RAW264.7 cells, which was blocked by N-acetyl-L-cysteine, an antioxidant. Our data suggest that PCT-induced HMGB1 release from macrophages involves endogenous H_2S generated by CSE, contributing to PIPN in mice.

1-SS-65

In vitro and *in vivo* pharmacology of YNT-X, a novel small-molecule orexin type 2 receptor agonist

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Orexin, a neuropeptide produced by neurons in the lateral hypothalamus, regulates sleep and wakefulness. Orexin deficiency causes narcolepsy-cataplexy characterized by excessive sleepiness and cataplexy, a sudden loss of muscle tone triggered by emotion.

We previously showed that peripherally (i.p.) administered YNT-185 (40 mg/kg), an OX2R-selective agonist, ameliorates narcolepsy-cataplexy symptoms in mouse models. However, repeated i.p. of YNT185 can be stressful and the effective dose for oral administration (p.o.) is too high (4000 mg/kg) to ameliorate the symptoms. Here we further optimized YNT-185 ($EC_{50} \approx 28$ nM) and produced YNT-X, which increased intracellular Ca^{2+} in lower concentrations in OX2R-transfected cells. The EC_{50} value was 1.1 nM (OX2R) and the maximum response was similar to orexin *in vitro.* The response was inhibited by EMPA, an OX2R antagonist. In vivo, YNT-X p.o. increased wake time in wildtype mice in a dose-dependent manner, with the effective dose of 2.5-10 mg/kg, which is several hundred times lower than YNT-185. Our results suggest that YNT-X may be useful as mechanistic, oral therapy for narcolepsy-cataplexy.

1-SS-66

Optogenetic inhibition of central serotonergic neurons impairs model-based decision making

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It has been speculated that serotonin release in the forebrain is involved in model-based decision making. However, there is so far no direct evidence proving this hypothesis because there had been no method that selectively controls serotonergic activity. To resolve this problem, we developed transgenic mice expressing Archaerhodopsin T (ArchT) only in central serotonergic neurons. A lithium devaluation task was used to assess model-based decision making. In this paradigm, a mouse is first trained to poke its nose to illuminated holes to get a food pellet, and then the food is devalued by pairing it with lithium-induced illness. If the mouse associates the devaluation with nose-poking by mental simulation though the mouse has never experienced these two events simultaneously, the mice will refrain from poking its nose to holes (i.e. model based-decision making). Our results indicated that optogenetic silencing of serotonergic neurons in the dorsal raphe nucleus, but not the median raphe nucleus, impaired model-based decision making. Thus it is likely that serotonergic activity in the dorsal raphe nucleus has a pivotal role in model-based decision making.

1-SS-67

The roles of serotonin 5-HT_{2C} receptor in locomotor activity, anxiety, and fear memory

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Pharmacological studies have suggested that serotonin 5-HT_{2C} receptor is involved in locomotor activity, anxiety, and fear memory. However, the results of locomotor activity and anxiety in 5-HT_{2C} receptor knockout mice are mixed, and the effects of 5-HT_{2C} receptor knockout on fear memory have not yet been addressed. In the present study, we reconciled these inconsistent results by analyzing behavioral data in details. We revealed that the higher locomotor activity in 5-HT_{2C} receptor knockout mice is observed only in the late phase of the test. Moreover, we found that 5-HT_{2C} receptor knockout mice display a hesitating attitude, staying in the center area and risk assessment behavior, in the elevated plus maze test. This phenotype might explain the inconsistency of previous studies. In the contextual fear conditioning test, 5-HT_{2C} receptor knockout mice tended to show rapid within-session extinction of fear, but not between-session extinction, compared to the wild type mice.

1-SS-68

Characterization of cells in the ventral tegmental area activated by abused drugs

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Characterization of cells in the ventral tegmental area activated by abused drugs

Generally, activation of the mesolimbic dopaminergic system, which projects from the ventral tegmental area (VTA) to the nucleus accumbens (N.Acc.), plays an important role in the development of psychological dependence on drugs of abuse. Medium spiny neurons in the N.Acc., which include dopamine D_1 receptors and mostly project to the VTA, are likely to be associated with relapse in drug abuse. Abused drugs have been classified as "uppers" and "downers," and it is believed that they cause drug addiction via different networks. In this study, we characterized cells in the VTA that are activated by abused drugs. In cFos-EGFP-Rp110a (cFos-TRAP) mice, drug-activated cells and non-activated cells were analyzed by FACS. As a result, most of the neurons in the VTA that were activated by the administration of either morphine or EtOH expressed tyrosine hydroxylase, dopamine transporter and mTOR. These results suggest that abused drugs may selectively activate dopaminergic neurons containing mTOR in the VTA. We are currently trying to identify the neurons that are activated by methylphenidate, benzodiazepines and other abused drugs to elucidate a common mechanism of drug addiction. This approach may help us understand the complex mechanism of psychological dependence on abused drugs.

1-SS-69

The deficit of quinolinic acid phosphoribosyltransferase induces hypolocomotion and cognitive impairment through impairment of dopaminergic neuronal function

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Quinolinic acid (QA) is a neurotoxic and implicated in the neurological disorders. QA is metabolized by quinolinic acid phosphoribosyltransferase (QPRT). However, the physiological roles of QPRT in central nervous system are still unclear. To investigate the roles of QPRT in emotional and cognitive functions, QPRT KO mice were subjected to several types of neurobehavioral tests. The KO mice decreased locomotor activity in novel environment, prolonged escape latency in Barns maze test, and decreased alternation behavior in Y-maze test. In the KO mice, the contents of homovanillic acid (HVA) and 3,4-Dihydroxyphenylacetic acid (DOPAC), and the ratios of HVA/ dopamine (DA) in the nucleus accumbens and DOPAC/DA in the prefrontal cortex were decrease. In the immunohistochemistry, the number of tyrosine hydroxylase (TH)-positive neuron was less compared with wild mice. Taken together, the present findings suggest a novel role of QPRT in hypolocomotion and cognitive impairment in relation to impairment of dopaminergic functions in the nucleus accumbens and prefrontal cortex, respectively.

1-SS-70

The transcription factor Npas4 regulates reward-related learning and memory

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Dopamine (DA) is necessary for motor function, motivation, working memory, and reward. However, how DA regulates reward-related learning and memorythrough the gene expression is not fully understood. Neuronal Per Arnt Sim domain protein 4 (Npas4), a brain-specific basic helix-loop-helix transcription factor, plays a role in synaptic plasticity by regulating the expression of activity-dependent genes,such as BDNF.Although Npas4 required forcontextual fear memory formation in mice, the role of Npas4 in reward-relatedlearning and memoryremains unknown. To this purpose, we used the conditioned place preference (CPP) paradigm in which animals learn to prefer a context associated with cocaine. Here, we found that the deletion of Npas4 in the accumbal D1R-expressing MSNs (D1R-MSNs) suppressedCPP. This phonotype was rescued by the D1R-MSNs specific expression of Npas4-WT but not phospho-deficient Npas4 mutant. These results suggestthat Npas4 and its phosphorylation regulate reward-related learning and memory in the accumbal D1R-MSNs.

1-SS-71

Analysis of molecular mechanism underlying cell death induced by an ALS/FTD-causative gene CHCHD10, S59L-CHCHD10

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Amyotrophic lateral sclerosis (ALS) is a motor neuron-specific neurodegenerative disease and frontotemporal dementia (FTD) is a neurodegenerative disease with young-onset dementia. Accumulating evidence indicates that they have common clinical and pathologic features. Several mutations of the *CHCHD10* (C10) gene have been found to cause ALS/FTD. Wild-type C10 is localized at mitochondria and physiologically involved in the regulation of mitochondrial function. It has been previously shown that a mutant C10 induces mitochondrial dysfunction such as the reduction in ATPase production that has been hypothesized to be closely linked to the ALS/FTD onset. In the present study, we have investigated the molecular mechanism underlying neuronal cell death caused by a mutant C10, S59L-C10. We have found that the adenovirus-medicated overexpression of wild-type C10 and S59L-C10 induces cell death *in vitro*. As expected, S59L-C10-induced cell death was more prominent than that induced by wild-type C10. This result suggests that S59L-C10-induced cell death may be caused by the gain-of-toxic mechanism. We have further characterized the pathway in detail underlying the S59L-C10-induced cell death.

1-SS-72

Fatty acid-binding protein ligands inhibit α-synuclein pathology in Parkinsonian mice

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[Background] Aggregation of α-synuclein (αS) is promoted by polyunsaturated fatty acids such as arachidonic acid (AA). Fatty acid-binding protein 3 (FABP3) is crucial for AA transport in the brain. We previously reported that FABP3 aggravates AA-induced αS oligomerization and cell death (Shioda et al, J Biol Chem, 2014). We here developed novel FABP3 ligands, MF series, and addressed whether these ligands could suppress αS pathology and Parkinsonism in mice.
[Methods] Mice treated with 1-methyl-1,2,3,6-tetrahydropiridine (MPTP) were chronically administrated with MF1 (high affinity for FABP3), MF4 (low affinity for FABP3), or L-DOPA.
[Results] MPTP-induced motor deficits were ameliorated by MF1 like L-DOPA. On the other hand, αS accumulation in dopaminergic (DA) cell bodies and neuronal loss in the substantia nigra (SN) of MPTP-treated mice were ameliorated by MF1, but not by L-DOPA nor MF4. Finally, MF1 also inhibited αS oligomerization and hyper-phosphorylation in the SN/VTA of MPTP-treated mice.
[Conclusion] MF1 but not L-DOPA suppressed αS pathology, thereby rescuing MPTP-induced DA neuronal loss and Parkinsonism. We propose novel FABP3 ligands as a candidate for synucleinopathies.

1-SS-73

The relevance between alteration intracellular potassium levels and mitochondrial depolarization-induced neurotoxicity.

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ATP-sensitive potassium channel (KATP) is a kind of inwardly rectifying potassium channel that suppresses depolarization in both cell membrane and mitochondria, and has an important role to make and keep resting membrane potential. It is known that activation of KATP protected the cells from ischemic damage in heart and brain. However, the underlying mechanism is not clear. Here, we have investigated that how minoxidil suppresses ischemic damage and excitotoxicity. Transient ischemia model mice were prepared by 1-h middle cerebral artery occlusion using 6-week old male C57/BL mice. Injection of minoxidil immediately after the operation prevented the damage in a concentration-dependent manner. N-methyl-D-aspartic acid (NMDA) induced mitochondrial depolarization after the increase in calcium influx into the cells. Increasing mitochondrial depolarization correlated to extent of neuronal degeneration, while pre-treatment of minoxidil inhibited it. Therefore, these results suggested that the decrease in intracellular potassium level may suppress the neurodegeneration via suppression of mitochondrial depolarization.

1-SS-74

Possible involvement of DNA methylation by DNMT3a in ischemia-induced neuronal injury

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DNA methylation of promoter region is thought to be involved in pathogenesis of several diseases. In cerebral ischemia, some studies have reported that infarct volume is decreased by inhibition of DNA methyltransferases (DNMTs) that mediates DNA methylation. However, roles of DNMTs in cerebral ischemia have not been clarified. Therefore, we investigated whether DNA methylation was associated with pathology of cerebral ischemia.

In this study, an *in vivo* cerebral ischemia was produced by occlusion of middle cerebral artery and reperfusion (MCAO/R) in the rat. First, we investigated protein levels of DNMTs. Protein levels of DNMT3a, but not DNMT3b, were increased in penumbra regions 1 day after MCAO/R compared with those in same regions of sham-operated rats. Also, Immunohistochemical examination 1 day after MCAO/R revealed that DNMT3a-positive cells in penumbra regions were colocalized with NeuN-positive neurons. Therefore, we determined effect of DNMTs inhibitor RG108 against *N*-methyl-D-aspartate (NMDA)-induced neuronal cell death in primary cultured rat cortical neurons. RG108 protected neurons from NMDA-induced cell death.

These results suggested that neuronal cell death after cerebral ischemia was correlated with DNA methylation by DNMT3a.

1-SS-75
Effects of furin inhibitors on excitotoxic neuronal damage

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Ischemic neuronal damage is induced by various factors such as glutamate excitotoxicity and oxidative stress. Excessive amount of extracellular glutamate followed by cerebral ischemia is a major factor to lead intracellular Ca^{2+} overload through the *N*-methyl-D-aspartate (NMDA) receptor and then causes neuronal death. It has been known that several proteolytic enzymes are stimulated by intracellular Ca^{2+} elevation. However, roles of proteolytic enzymes on NMDA-induced neuronal damage are not fully defined. We examined whether inhibitions of proteolytic enzymes protected primary cultured rat cortical neurons from NMDA-induced damage. Among several inhibitors, furin inhibitor markedly protected neurons from NMDA-induced damage in a dose-dependent manner. Furthermore, calpain activation led by NMDA treatment was inhibited by the furin inhibitor. We next investigated the ability of furin inhibitor to attenuate cell damage in a rat cerebral ischemia model. We demonstrated that infarct volume after cerebral ischemia was not affected by treatment with furin inhibitor. Although further research will be needed to assess the therapeutic potential of furin inhibitor in cerebral ischemia *in vivo*, this study revealed a novel role of furin in excitotoxic injury in cortical neurons.

1-SS-76

Analysis of onset mechanism for lithium carbonate-induced cardiovascular adverse events assessed in the halothane-anesthetized dogs

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Introduction: Lithium has been widely used for the treatment of bipolar disorder. However, several cardiac adverse events have been noticed in patients who were also treated with other drugs in addition to lithium. In the present study, we studied the effects of lithium by itself to precisely analyze the onset mechanisms of its cardiovascular adverse events.

Methods: We intravenously administered lithium carbonate in doses of 0.1, 1 and 10 mg/kg/10 min to the halothane-anesthetized dogs (n=4) under the monitoring of cardiohemodynamic and electrophysiological variables.

Results: The currently used doses of lithium provided plasma concentrations ranging from sub-therapeutic to toxic ones. The low and middle doses significantly prolonged the ventricular effective refractory period. Additionally, the high dose significantly decreased the heart rate, delayed the intraventricular conduction and the ventricular repolarization, and kept the effective refractory period prolonged.

Conclusion: Lithium may have a wide safety margin against hemodynamic adverse events excpt that it can moderately inhibit both Na^+ and K^+ channels, leading to increase of the ventricular refractoriness and decrease of the heart rate.

1-SS-77

Uroguanylin affects the calcium current in a complicated manner

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[Background] It is reported that natriuretic peptides affect the action potential and I_{CaL} in cardiomyocytes via an increase in cGMP by activation of particulate guanylate cyclase (pGC). Uroguanylin (uro) is known to increase cGMP through pGC. In this study, we examined the effects of uro on I_{CaL} in cardiomyocytes.

[Methods] Ventricular cells were isolated from guinea-pig hearts. The I_{CaL} was recorded by use of whole-cell patch clamp method.

[Results] When uro was applied to the myocytes while raising its concentration (3 nM〜0.3 μM) after stimulating I_{CaL} by 0.3 μM isoproterenol (iso), the stimulated- I_{CaL} was inhibited by uro on the whole. However, uro sometimes augmented the stimulated- I_{CaL}. The basal I_{CaL} was not affected by uro. ODQ, a selective soluble GC inhibitor, 0.1 mM did not exert the inhibitory effect on the action of uro. Rp-8-Br-PET-cGMPs (Rp), one of the most potent cGMP-PKG inhibitor, 10 μM in patch pipette did not affect either, however, the uro-evoked augmentation of the stimulated- I_{CaL} was not observed in the presence of Rp.

[Conclusion] Uroguanylin exerted the inhibitory effects on the iso-stimulated I_{CaL} in a lot of cases, but its effects were not consistent. The mechanism of the uro-induced activation of pGC and I_{CaL} remained to be elucidated

1-SS-78

Monensin-induced Ca^{2+} overload suppresses mitochondrial ATP production in cardiac myocytes

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[Background]
Monensin (Mo) has been reported to decrease the ATP content in various cells, however, its mechanisms are not fully understood in cardiac myocytes. In this study, the effects of Mo on ATP production was examined in guinea-pig ventricular myocytes.
[Methods]
The ATP production was evaluated from the time taken to open sarcolemmal K_{ATP} channel. Membrane potential and Ca^{2+} concentration of mitochondria (mito) was measured.
[Results]
When the extracellular solution containing 112 mM Na,o and the intracellular solution containing 10 mM Na,i and 0 mM ATP were used, Mo 10^{-5} M shortened time taken to open the K_{ATP} channel significantly. When 0 mM Na,o and 10 mM Na,i were used, Mo also shortened time. Next, when 0 mM Na,o and 0 mM Na,i were used, Mo shortened the time to a lesser extent. When EGTA 5 mM was replaced by BAPTA 40 mM in the patch pipette, the time taken to open the K_{ATP} channel was not shortened so much by Mo. Mo increased mito Ca^{2+} and depolarized the membrane potential in saponin-treated myocytes.
[Conclusion]
We conclude that the Mo-induced Na^{+} influx into mito alters Na^{+}/ Ca^{2+} exchange function, and the mito membrane depolarization may cause Ca^{2+} influx to mito matrix, leading to suppression of ATP production.

1-SS-79

Effect of electrical field stimulation on intracellular sodium concentration in human iPS cardiomyocytes

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Human-induced pluripotent stem cell-derived cardiomyocytes (hiPS-CMs) are significantly immature compared with adult cardiomyocytes and exhibit a fetal-like phenotype. Although efficient propagation of electrical signals in the heart is crucial for natural development program and functionality, maturation of hiPS-CMs causes hyperpolarization of resting membrane potentials and a loss of automaticity *in vitro*. We therefore investigated whether artificial electrical field stimulations mature physiological properties of hiPS-CMs, especially on intracellular sodium concentrations ($[Na^+]_i$). We cultured hiPS-CMs for a week under electrical field stimulations (3 V/cm, 1 ms, 1 Hz), and quantified $[Na^+]_i$ with a Na^+ indicator. Basal intracellular sodium concentration without stimuli was 4.7 mM (n = 17) which is much lower than that in normal excitable cells (10-15 mM). The intracellular sodium concentration with the electrical stimuli (5.7 mM, n = 23) was significantly greater than $[Na^+]_i$ without stimuli. These results suggest that artificial electrical stimulations enable us to obtain more mature hiPS-CMs *in vitro*.

1-SS-80

Reciprocal action of a synthetic estrogen and hERG blockers on the hERG channel

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Inhibition of K^+-conductance through the hERG channel leads QT prolongation and associates with cardiac arrhythmias. We have found that estrogens interact with a drug-binding site, F656 of the hERG channel, and alter effects of a hERG blocker. However precise mechanistic insights have not been elucidated. We here investigated actions of ethynylestradiol (EE2), a synthetic estrogen, on the hERG channel. HEK293 cells stably-expressing the hERG channels were cultured in the steroid-free medium. The patch-clamp technique is performed to record hERG currents. Supratherapeutic concentrations of EE2 did not alter amplitudes and kinetics of the hERG currents elicited with train pulses at 20 mV (0.1 Hz). On the other hand, EE2 recovered the hERG inhibition induced by E-4031. These results suggest that EE2 interacts with E-4031 at the promiscuous drug-binding site of the hERG channel and imply that EE2, an anti-breast cancer drug or an oral contraceptive, is protective against drug-induced QT prolongation.