

1-P-001

p11 in cholinergic interneurons of the nucleus accumbens is essential for dopamine responses to rewarding stimuli

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The present study investigated the role of p11 in NAc CINs in dopamine responses to rewarding stimuli (cocaine, palatable food and female mouse encounter). The extracellular dopamine and acetylcholine (ACh) levels in the NAc were determined in freely moving male mice using *in vivo* microdialysis.

Rewarding stimuli induced an increase in dopamine efflux in the NAc of wild-type mice. The dopamine responses were attenuated in constitutive p11 KO mice. The dopamine response to cocaine was accompanied by an increase in ACh NAc efflux, whereas the attenuated dopamine response to cocaine in p11 KO mice was restored by pharmacological activation of ACh receptors in the NAc.

Dopamine response to cocaine and an increase in ACh were attenuated in mice with deletion of p11 from cholinergic neurons (ChAT-p11 cKO mice), whereas gene delivery of p11 to CINs and chemogenetic activation of CINs restored the dopamine responses.

Thus, p11 in NAc CINs plays a critical role in activating these neurons to mediate dopamine responses to rewarding stimuli. The dysregulation of mesolimbic dopamine system by dysfunction of p11 in NAc CINs may be involved in pathogenesis of depressive states.

1-P-002

Accumbal GABA_A and GABA_B receptors each inhibit acetylcholine efflux without affecting dopamine efflux in the nucleus accumbens of freely moving rats

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We analyzed the roles of GABA_A and GABA_B receptors (-Rs) in regulating acetylcholine (ACh) efflux in the nucleus accumbens (NAc) of freely moving rats using *in vivo* microdialysis. The effects of GABA-R ligands on accumbal dopamine (DA) efflux were also analyzed as accumbal cholinergic and dopaminergic neurons could mutually interact. Drugs used were infused directly into NAc. The GABA_A-R agonist muscimol (30 pmol) and the GABA_B-R agonist baclofen (300 pmol) each reduced accumbal ACh. The GABA_A-R antagonist bicuculline (60 pmol) counteracted the muscimol (30 pmol)-induced decrease in ACh and the GABA_B-R antagonist saclofen (12 nmol) counteracted the baclofen (300 pmol)-induced decrease in ACh. Each of muscimol (30 pmol), baclofen (300 pmol), bicuculline (60 pmol) and saclofen (12 nmol) did not alter baseline accumbal DA when given alone. Doses of compounds indicate total amount infused (mol) during 30-60 min infusions. These results show that GABA_A and GABA_B-Rs exert inhibitory roles in the control of accumbal ACh efflux. This study provides *in vivo* evidence that GABA_A and GABA_B-Rs each reduce accumbal cholinergic activity without affecting accumbal dopaminergic activity.

1-P-003

Enhancement of 5-HT synthesis and metabolism in aspartoacylase-knockout rats.

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Essential tremor (ET) is one of the most common movement disorders, exhibiting a postural and/or kinetic tremor. We previously analyzed tremorgenic gene components using a ET model, Tremor rats, and showed that deletion of aspartoacylase (*Aspa*) and a missense mutation of *Hcn1* channels are implicated in induction of tremor (Exp. Anim. 65, 293–301, 2016). Since monoamines are known to be involved in regulation of tremor induction, here, we evaluated the changes in brain monoamine levels in *Aspa*-knockout (KO) rats. Dopamine, 5-HT and their metabolites were analyzed in 9 brain regions (cerebral cortex, hippocampus, striatum, thalamus, hypothalamus, midbrain, inferior olive, pons and cerebellum), using a HPLC-ECD method. *Aspa*-KO rats showed increases in 5-HT levels in the hypothalamus and inferior olive, and in 5-HIAA and 5-HIAA/5-HT levels, in most brain regions examined, indicating that deletion of *Aspa* gene enhances the 5-HT turnover rates. Alterations in HVA levels were also found some regions (increases: cortex and striatum, decreases: hippocampus and hypothalamus). The present results suggest that enhancement 5-HT synthesis and metabolism, especially in the inferior olive, may be linked to tremor induction.

1-P-004

5-HT-induced $\text{Ca}_v2.1$ dependent inhibition of excitatory transmission onto cholinergic neurons in basal forebrain

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Cholinergic neurons in basal forebrain (BF) project to various brain regions including cortex and hippocampus. BF receives various inputs such as serotonergic fibers from the dorsal raphe nuclei. However, serotonin (5-HT)-induced modulatory effects on the excitatory synaptic transmission in BF are unknown. This study was aimed to elucidate 5-HT-induced modulation of glutamatergic synaptic transmission onto BF cholinergic neurons. BF cholinergic neurons were identified with Cy3-192IgG and investigated in P12-20 rat brain slices. Excitatory postsynaptic currents (EPSCs) were evoked by focal electrical stimulation. 5-HT, a 5-HT_{1A} receptor agonist or a 5-HT_{1B} receptor agonist inhibited the amplitude of EPSCs. In the presence of both 5-HT_{1A} and 5-HT_{1B} receptor antagonists, most of 5-HT-induced effect disappeared. 5-HT-induced inhibition was significantly smaller in the presence of ω -agatoxin TK than that without ω -agatoxin TK. 5-HT reduced synaptic strength by changing AMPA/ NMDA ratio. These results suggest that 5-HT inhibits glutamatergic transmission onto BF cholinergic neurons via both 5-HT_{1A} and 5-HT_{1B} receptors. They also suggest that 5-HT inhibits glutamatergic transmission by selectively blocking CaV2.1 (P/Q-type).

1-P-005

Deletion of brain histidine decarboxylase by adeno-associated virus induced anxiety-like behaviors in adult mice.

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Histamine acts as a neurotransmitter in the brain. Histamine is synthesized from histidine by catalyzing histidine decarboxylase (HDC). In the central nervous system, HDC-positive neurons are in tuberomammillary nucleus of posterior hypothalamus and project their axons to entire brain. However, the roles of HDC in brain functions are not fully elucidated. In the present study, we generated mice with brain-specific deletion of HDC to investigate the importance of histamine for adult brain.

We stereotactically microinjected adeno-associated virus (AAV) expressing Cre-recombinase into tuberomammillary nucleus of adult HDC flox mice (HDC cKO mice). Immunohistochemical analysis showed Cre expression in tuberomammillary nucleus in HDC cKO mice. We confirmed the reduced HDC mRNA expression and the decreased histamine content in HDC cKO brain. Light/dark box tests showed that HDC cKO mice spent a shorter amount of time in the light room. In the tail suspension tests, immobility time was prolonged in HDC cKO mice. These results indicated that the inhibition of HDC activity in adult brain reduced histamine content and induced anxiety- and depression-like behaviors in mice.

1-P-006

Drug development targeting histamine *N*-methyltransferase

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Brain histamine has a role as a neurotransmitter in the regulation of various physiological functions. Previous studies revealed the involvement of histamine depletion in several neurological disorders, leading to the development of new drugs targeting brain histamine system. Histamine *N*-methyltransferase (HNMT), a histamine metabolizing enzyme, had a great impact on brain histamine concentration, assuming that HNMT inhibition can increase histamine and have a therapeutic effect on various brain diseases. Here, we aimed to reveal the HNMT functions and find new HNMT inhibitors to activate brain histamine system and improve brain functions.

First, we phenotyped HNMT knockout mice. HNMT deficiency increased the amount of brain histamine, indicating the essential role of HNMT in brain histamine. Next, we constructed high-throughput screening (HTS) system to find HNMT inhibitors. Six thousands compounds were applied to the system and 9 compounds (A-I) were selected as candidates. Direct administration into hypothalamus and systemic administration of compound G could increase extracellular histamine. These results demonstrated that compound G was a potential candidate as a new HNMT inhibitor.

1-P-007

Gabapentin enhances glutamate-induced glutamate release from cultured astrocytes

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We have recently demonstrated that local injection of glutamate transporter activator riluzole (RIL) and gabapentin (GBP) into the locus coeruleus activated noradrenergic neurons via glutamate signalling and resulting descending pain inhibition in rats, indicating that both RIL and GBP may share common mechanisms in astrocytes. In the present study, we examined the effects of RIL and GBP on glutamate uptake/release and glutamate-induced Ca^{2+} response in cultured astrocytes. Pre-treatment with RIL or GBP significantly increased glutamate uptake which was completely blocked by a non-selective glutamate transporter blocker, DL-TBOA. The enhancement of glutamate-induced intracellular Ca^{2+} response by RIL or GBP was blocked by the DL-TBOA and an inhibitor of $\text{Na}^+/\text{Ca}^{2+}$ exchanger, KB-R7943. Glutamate release from cultured astrocytes significantly increased in the presence of RIL and GBP. These results suggest that RIL and GBP enhance Na^+ -glutamate co-transport through glutamate transporters, and subsequent Ca^{2+} influx via the reverse mode of $\text{Na}^+/\text{Ca}^{2+}$ exchange enhances glutamate-induced glutamate release in astrocytes. The present study also suggests that GBP have a novel target of action in astrocytes other than $\alpha_2\delta$ subunits.

1-P-008

CNB-001, a synthesized pyrazole derivative of curcumin, induces astrocyte stellation through activation of calcium/calmodulin-dependent protein kinase II

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CNB-001, a pyrazole derivative of curcumin, has a variety of biological effects that may be beneficial for the treatment of neurodegenerative disorders including Alzheimer's disease. To further explore CNB-001 as a potential therapeutic drug, we investigated its effect on the morphology of astrocytes. Cultured rat cortical astrocytes exhibited flat, polygonal morphology in the absence of stimulation. When the cells were treated with CNB-001 (10 μ M), they changed into process-bearing stellate cells. Since curcumin (10 μ M) had no effect on astrocyte morphology, the stellation-inducing effect is likely to be specific for CNB-001. MTT reduction assay demonstrated that CNB-001 (10 μ M) had no effect on the cell viability. The CNB-001-induced astrocyte stellation was inhibited by the presence of KN-93 (10 μ M), an inhibitor of calcium/calmodulin-dependent protein kinase II (CaMKII), but not KN-92 (10 μ M), an inactive analog of KN-93. These results suggest that CNB-001 induces astrocyte stellation through the activation of CaMKII.

1-P-009

Search for prophylactic or therapeutic agents for dementia (Alzheimer's type)

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The main objective of our study is to prevent dementia (Alzheimer's type). We examined the effects of pretreatment with the ether extract of natural plants on pathological changes due to domoic acid (DA) (Tsunekawa *et al.*, 2013). The natural plant that was effective in this preliminary experiment was *Graptopetalum paraguayense* (GP). The 50% effective dose was 0.15 mg/kg. A stronger positive response was observed in the hippocampal , parasubiculum (PaS), presubiculum (PrS) , and entorhinal cortex (Ent) after 5 days. After 50 days, strong staining appeared in the hippocampus, the apoptosis was induced. After 50 days, we recognized the deposition of amyloid β in the extracellular matrix between cells. We did not observe amyloid β deposition when the brain had been pretreated with GP extract. The GP-extract-pretreated group was tunel negative after 1 and 5 days, negative in the hippocampal area after 50 days, and positive in the PaS, PrS, and Ent. These results indicate that GP suppressed apoptosis in most cells, except for Pas, PeS, and Ent, in the hippocampal area. In addition, amyloid β deposition was also suppressed. These results suggest that GP suppresses the pathological changes that are commonly seen in the dementia.

1-P-010

Effects of nicotinic acetylcholine receptors on neurons, microglia, and bone marrow-derived microglia-like cells for the treatment of Alzheimer's disease.

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The accumulation of amyloid- β peptides ($A\beta$) is a critical trigger of Alzheimer's disease (AD) pathogenesis. $A\beta$ is derived from sequential proteolysis of amyloid precursor protein by β - and γ -secretases. Nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels. Microglia and bone marrow-derived microglia-like (BMDML) cells have $A\beta$ phagocytic function, and nicotinic stimulation promotes microglial $A\beta$ phagocytosis. However, it is not yet known the nicotinic effects on BMDML cells and nAChR subtypes which involve in the promotion of microglial $A\beta$ phagocytosis. We here found that the stimulation of nAChRs promoted $A\beta$ phagocytosis in BMDML cells and $\alpha 7$ nAChR was involved in the promotion of microglial $A\beta$ phagocytosis. In a mouse model of AD, stimulation of $\alpha 7$ nAChR attenuated brain $A\beta$ burden and memory dysfunction. Moreover, $\alpha 7$ nAChR agonist suppressed γ -secretase activity. Results suggests that $\alpha 7$ nAChR subtype is a reasonable drug discovery target in AD, and the stimulation of nAChRs in BMDML cells may be a beneficial option for the development of the cell therapy in AD.

1-P-011

Microglia promote the proliferation of neural precursor cells by secreting osteopontin

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Microglia are resident immune cells of the central nervous system, and play crucial roles in brain development by regulating the number of neural precursor cells. Recent study showed microglia surrounding the subventricular zone express osteopontin (OPN) during brain development. OPN is a phosphoglycoprotein involved in immune responses, cancer metastasis, and cell proliferation. However, it remains unclear whether OPN in microglia is involved in brain development. In this study, we investigate the contribution of OPN secreted from microglia to the proliferation of neural precursor cells. We examined the expression of integrin $\alpha\beta3$, and CD44, which are receptors for OPN, in neural precursor cells, and found that neural precursor cells express integrin $\alpha\beta3$, but not CD44. To examine whether microglia promote the proliferation of neural precursor cells by OPN-integrin $\alpha\beta3$ signaling, we performed co-culture experiment of microglia and neural precursor cells across the transwell in the presence of OPN neutralizing antibody or cilengitide, an inhibitor for integrin $\alpha\beta3$. Our study indicates signaling between OPN and integrin $\alpha\beta3$ is a crucial contributor to regulate the proliferation of neural precursor cells mediated by microglia during brain development.

1-P-012

Effect of Sema4D on proliferation and L-arginine metabolism in ischemic microglia

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Cerebral ischemia induces massive neuroinflammation and microglial activation. Activated microglia change their phenotype, including metabolism. Inducible nitric oxide synthase (iNOS) and arginase-1 (Arg1) are expressed in activated microglia, and they competitively metabolize L-arginine to NO or precursor of polyamines, respectively. We have reported that a class 4 semaphorin (Sema4D) deficiency inhibits microglial iNOS expression and NO production and promotes microglial proliferation in cerebral ischemia, although underlying mechanisms remain unclear. In this study, we found that Sema4D deficiency alters the balance of competition between iNOS and Arg1, and pushes from NO-production to polyamines-production in microglia. Furthermore, polyamine putrescine promoted proliferation of cultured microglia. Our findings indicate that Sema4D regulates microglial proliferation via L-arginine metabolic pathway in the ischemic brain.

1-P-013

Transcription factor MafB contributes to activation of spinal microglia and neuropathic pain after peripheral nerve injury

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Microglia, which are pathological effectors and amplifiers in the central nervous system, undergo various forms of activation. Activation of spinal microglia following peripheral nerve injury (PNI), is a key event for the development of neuropathic pain but the transcription factors contributing to microglial activation are less understood.

Here, we demonstrate that MafB, a dominant transcriptional regulator of mature microglia, is involved in the pathology of neuropathic pain. PNI caused an increase of MafB expression selectively in spinal microglia. We measured expression of mir-152, a microRNA targeting MafB, and found a transient decrease in its expression in the spinal cord after PNI. Moreover, intrathecal administration of mir-152 mimic suppressed the development of neuropathic pain. Reduced MafB expression using heterozygous Mafb-deficient mice alleviated mechanical hypersensitivity. Furthermore, we found that intrathecal transfer of Mafb-deficient microglia did not induce mechanical hypersensitivity and that conditional Mafb-knockout mice did not develop neuropathic pain. We propose that MafB is a key mediator of the PNI-induced phenotypic alteration of spinal microglia and neuropathic pain development.

1-P-014

CNB-001, a synthesized pyrazole derivative of curcumin, inhibits thrombin-induced nitric oxide production in primary cultured rat microglia

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We have recently found that CNB-001, a pyrazole derivative of curcumin, suppresses lipopolysaccharide (LPS)-induced nitric oxide (NO) production in cultured microglia, demonstrating that it exerts anti-neuroinflammatory effects by regulating microglial activation. The present study was undertaken to investigate whether CNB-001 is also effective for microglial activation induced by other stimulants than LPS. Treatment of primary cultured rat microglia with thrombin (0.1-30 U/mL), a serine protease that has been proposed as a mediator of cerebrovascular injuries, caused the NO production in a concentration-dependent manner. Time-dependent accumulation of NO production was also detected in microglia treated with thrombin (10 U/mL) in 0-48 h, but not in untreated cells. CNB-001 (0.1-10 μ M) suppressed the NO production in a concentration-dependent manner. Western blotting analysis demonstrated that thrombin (10 U/mL) induced the inducible NO synthase (iNOS) expression in the cells, which was markedly inhibited by the presence of CNB-001 (10 μ M). These results suggest that CNB-001 exerts anti-inflammatory effects by inhibiting iNOS-mediated NO production in microglia.

1-P-015

TRPV4 activation enhances LPS-induced IL10 production to suppress excessive microglial activation

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Under pathological conditions, microglia trigger neurodegeneration by secreting pro-inflammatory molecules, whereas they possess a negative feedback mechanism involving anti-inflammatory cytokines such as IL10. We have previously shown that activation of transient receptor potential vanilloid 4 (TRPV4), a Ca^{2+} -permeable cation channels that sense hypo-osmolarity, mechanical stimulus and warm temperature, suppresses LPS-induced microglial activation. Here, we examined whether TRPV4 contributes anti-inflammatory responses using cultured murine microglia and found a selective TRPV4 agonist GSK1016790A enhanced LPS-induced IL10 production, which was fully suppressed by co-application of a selective TRPV4 antagonist GSK2193874 or TRPV4 gene deletion. Furthermore, neutralization of IL10 significantly reversed the inhibitory effects of GSK1016790A on LPS-induced production of pro-inflammatory cytokines. Expression pattern of activation markers implied that GSK1016790A shifted in microglial activation status to an immunoregulatory phenotype. Taken together, these results indicate that microglial TRPV4 plays an important role in promoting the production of LPS-induced IL10, which results in suppressing excessive inflammation in an autocrine or paracrine fashion.

1-P-016

Activation of toll-like receptor 4 induces downregulation of $\alpha 7$ nicotinic acetylcholine receptor in microglia

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Background: Inflammatory responses could be involved in induction of Alzheimer's disease (AD). Microglia are known to act as the main immunologic effector cell in the central nervous system, and contribute to the pathological states of AD. Activation of $\alpha 7$ nicotinic acetylcholine receptor ($\alpha 7$ -nAChR) potentiated microglial phagocytosis of amyloid- β . By contrast, $\alpha 7$ -nAChR was decreased along with the progression of AD in rodent models. The current study has investigated the mechanisms underlying downregulation of microglial $\alpha 7$ -nAChR expression by activation of toll-like receptor 4 (TLR4).

Method: Mice microglial cell-line BV2 cells were used for investigation of downregulation of $\alpha 7$ -nAChR expression. The mRNA expression levels of $\alpha 7$ -nAChR was measured by the real-time PCR.

Results: Treatment of BV2 cells with lipopolysaccharide (LPS) significantly decreased the expression of $\alpha 7$ -nAChR in dose- and time dependent manner. The LPS-induced downregulation of $\alpha 7$ -nAChR mRNA was mediated by TLR4 and histone deacetylase (HDAC), but not DNA methyltransferase.

Conclusion: The current study demonstrated that the TLR4-HDAC pathway might contribute to the downregulation of $\alpha 7$ -nAChR in microglia.

1-P-017

Deciphering the role of the histone methyltransferase SETD1A in Schizophrenia

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Schizophrenia (SCZ) is a severe, chronic psychiatric illness characterized by delusions and hallucinations, negative symptoms, and cognitive dysfunction that often lead to a lifetime of impairment and disability. Drug development for cognitive dysfunctions of patients with SCZ remains challenging, given the absence of a unifying pathophysiology, and the highly complex underlying genetic architecture. *SETD1A*, a histone methyltransferase, is a key schizophrenia susceptibility gene. To understand how *SETD1A* deficiency increases disease risk we employed a mouse model carrying a heterozygous loss-of-function mutation of the orthologous gene. We report that mutant mice exhibit alterations in axonal branching and working memory, as well as a molecular pathology pattern that recapitulates SCZ-related alterations. Notably, restoring *Setd1a* expression in adulthood rescues working memory deficits. Moreover, we identify demethylases counteracting the effects of *Setd1a* methyl transferase activity and show that the demethylase antagonism in *Setd1a*-deficient mice results in a full rescue of the behavioral abnormalities and axonal branching deficits. Our findings advance our understanding of how *SETD1A* mutations predispose to SCZ and other neurodevelopmental disorders and point to therapeutic interventions.

1-P-018

Acute treatment with cyclophosphamide in childhood suppresses hippocampal neurogenesis in the adulthood.

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Chemotherapy for childhood cancer can cause late-appearing side effects in survivors that affect multiple organs, including the brain. Cyclophosphamide is used in childhood cancer and has the blood brain barrier permeability. The present study aims to reveal whether an acute treatment with cyclophosphamide in childhood affects the mental function and hippocampal neurogenesis in the adult mouse. Cyclophosphamide (50, 100 or 200 mg/kg) were intraperitoneally once injected to 3 weeks old male ddY mice. Five weeks after injection, the behavioral test battery was performed. After the test battery, mice were received 2 consecutive injections of BrdU (50 mg/kg) with a 12-h interval. BrdU positive cells in the hippocampal dentate gyrus were counted after immunostaining for BrdU. Treatment with cyclophosphamide had the ability to decrease the number of BrdU-positive cells in the dentate gyrus 5 weeks afterward. However, cyclophosphamide failed to change emotional and cognitive functions. These data suggest that the acute treatment with cyclophosphamide in childhood exhibits the prolonged suppressive effect on hippocampal neurogenesis, but not affecting emotional and cognitive functions under physiological conditions.

1-P-019

Decreased hippocampal GLP-1 and the shift in the circadian rhythms are induced by long-term exposure of isoflurane with surgery in mice

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Inhalation anesthesia with isoflurane is occasionally associated with the postoperative cognitive impairment and shift in the circadian rhythms. The microglial activation is suggested to be pathogenesis of these complications. Glucagon-like peptide 1 (GLP-1) is specifically expressed in microglia and are associated with circadian transcription factor in hippocampus. We previously reported that hippocampal GLP-1 is reduced with cognitive dysfunction. Therefore, we investigated whether the hippocampal GLP-1 is affected by surgery with inhalation anesthesia. The spatial working memory subjected by spontaneous alteration in the Y-maze is significantly decreased 24 hours in mice with 2h-exposure of isoflurane plus abdominal surgery. In addition, circadian rhythms are significantly shifted during 7 days social isolation after the 2h-exposure of isoflurane with surgery. Western blotting revealed that the protein level of GLP-1 was decreased to the subdetection level for more than three days after the inhalation anesthesia with isoflurane plus abdominal surgery. These results indicate the possibility that the GLP-1 may contribute to the postoperative cognitive dysfunction and shift the circadian rhythms.

1-P-020

A ROCK inhibitor, fasudil, prevents behavioral changes induced by social defeat stress in mice

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Previously, we reported that expression of Rho/Rho-associated kinase (Rho/ROCK) pathway related genes in the rat brain were decreased after chronic administration of antidepressants, imipramine and sertraline. Then, we hypothesized that inhibition of ROCK would serve as a medication to treat stress-related mental disorders. In this study, we investigated the effect of a ROCK inhibitor, fasudil, using social defeat stress (SDS) mouse, which is considered as an animal model of stress-related mental disorders. As expected, SDS mice showed significant increase in the immobility compared with control mice in forced swim test. Interestingly, administration of fasudil during the stress exposure significantly decreased the immobility, while no significant effect was observed in SDS mice treated with fasudil after the stress exposure. Our results may suggest that fasudil would serve as a preventive medication for stress-related mental disorders. Since fasudil has already been used clinically for the treatment of cerebral vasospasm, clinical trials to examine the efficacy of fasudil in patients with stress-related mental disorders are warranted.

1-P-021

Mouse brain regions involved in ambulation promoted by *l*-menthol

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The present study aimed to explore mouse brain regions involved in ambulation promoted by *l*-menthol. Saline or 400 mg/kg *l*-menthol was subcutaneously administered to mice, and ambulatory activity was measured for 1 hour. The dose of *l*-menthol significantly promoted mouse ambulation. Immediately after the measurements, the animals were transcardially perfused with fixative and the brains were collected. The brains were subjected to c-Fos immunocytochemistry. The number of c-Fos like immunoreactivity in 24 brain regions was counted. The number of c-Fos like immunoreactivity in each of the brain regions was compared between *l*-menthol administered mice and saline administered mice. Relationship between the ambulatory activity and the number of c-Fos like immunoreactivity in the brain regions was examined using multiple regression analysis. Furthermore, relationships between the ambulatory activity and the number of c-Fos like immunoreactivity in each of the brain regions were examined using single regression analysis. These analyses indicated brain regions in which the number of c-Fos like immunoreactivity was related to ambulation promoted by *l*-menthol.

1-P-022

Analyzing task-related neural activity with two-photon calcium imaging from behaving mice

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Animals process and integrate sensory inputs to generate appropriate behavioral output. However, neural mechanisms underlying this process remain unclear. Here we established a short-term, visual discrimination task for head-fixed mice combined with *in vivo* two-photon imaging to relate neural activity to behavior. Viral vectors encoding the calcium indicator protein GCaMP6m were injected into the visual cortex of adult C57BL/6j mice. Water-restricted mice were trained to discriminate a target stimulus consisting of a grating drifting towards one horizontal direction, from a non-target stimulus drifting towards the opposite direction. The trained mice correctly responded to the target stimulus to lick a water-reward spout in front of the animal. After finishing the training, neural activity recording was carried out using two-photon calcium imaging from the mice performing the task. This head fixed, lick/no-lick visual discrimination task offers a quick learning paradigm compatible with pharmacological and neurophysiological methods including two-photon imaging, optogenetics and circuit tracing, making it possible to understand circuits and computations underlying perceptual decision-making as well as neurocognitive disorders.

1-P-023

Optimization of operant test for testing risky decision-making in mice

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Decision making in complex and uncertain situations is a fundamental adaptive process resulting from the integration of several executive functions. Impaired decision-making is a symptomatic feature of a number of psychiatric disorders. In general, patients with psychiatric disorders show a propensity to prefer actions associated with large short-term gains but long-term losses preferentially to those associated with small but long-term gains. They are more likely to select risky options and show an altered temporal horizon of risks and benefits. Moreover, clinical studies report that dopamine therapy induce impaired decision-making in patients with dopaminergic dysfunction such as Parkinson's disease and Redox-less syndrome. Thus, to establish and optimize the animal model of impaired decision-making is important for development of new therapeutic strategy in these disorders.

To clarify the underlying neurobiology of decision-making, we conducted a study in healthy mice by using a mouse gambling operant test based on uncertainty and conflicting choices. Mice chose the low-risk / low-reward option on the reward amount- and provability-dependent manner. Our findings suggest that healthy mice prefer risk-averse choice in this operant test as dose man in Iowa gambling test.

1-P-024

The pilocarpine seizure sensitivity test is capable of detecting masked late posttraumatic epilepsy after controlled cortical impact in mice

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Late posttraumatic epilepsy (PTE) following traumatic brain injury (TBI) is characterized by long and unexpected latencies until the onset of seizure. Interventions with antiepileptic drugs before seizure onset have not been found to prevent late-onset PTE. Greater understanding of the development of epileptogenicity underlying late PTE following TBI is needed to develop effective interventions at an appropriate time-point. Here we describe a simple behavioral evaluation method for detecting each stage in the development of epileptogenicity during the period from TBI until epileptic seizure occurrence in mice. Pilocarpine (250 mg/kg) was injected at different post-operative days into mice subjected to controlled cortical impact (CCI), a common model for experimental TBI. The seizure sensitivity test was then performed. Seizure severity and incidence at the pilocarpine sub-threshold dose gradually increased from 7 days after CCI, reaching a significant increase at 28 days after CCI. The proposed simple pilocarpine test in CCI mice is relevant for monitoring the development of epileptogenicity, and could help researchers develop methods for early diagnosis, prevention and treatment of PTE.

1-P-025

Beta oscillations in the mouse hippocampus during object location recognition

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Memories about the location of environmental cues are crucial for survival and are supported by a distributed network including the hippocampus and the retrosplenial cortex (RSC). The relationships between neuronal activity of these brain areas and object location memory are not fully understood. In this study, we monitored local field potential oscillations simultaneously from the hippocampus and the RSC in freely moving mice during the object-location task, a test to assess memories about the locations of environmental cues. In this task, mice were exposed to two identical objects in an open field and were allowed to explore objects and learn the location of each object. We discovered that immediately before explorations to the objects during memory encoding sessions, beta oscillations (23-30 Hz) transiently increased in the hippocampus and the RSC. Moreover, spectral coherence in the hippocampus-RSC circuit in the beta band was also enhanced before the explorations. Furthermore, mice with better memory showed greater enhancement of the beta power during memory encoding sessions. Our data suggest that enhanced beta oscillations facilitate memory acquisition about the location of landmarks.

1-P-026

Phosphorylation of Npas4 by MAPK regulates reward-related gene expression and behaviors

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Dopamine activates MAPK via PKA/Rap1 in medium spiny neurons (MSNs) expressing the dopamine D1 receptor (D1R) in the nucleus accumbens (NAc), thereby regulating reward-related behavior. However, how MAPK regulates reward-related learning and memory through gene expression is poorly understood. Here, to identify the relevant transcriptional factors, we performed proteomic analysis using affinity beads coated with CREB-binding protein (CBP), a transcriptional coactivator involved in reward-related behavior. We identified more than 100 CBP-interacting proteins, including Neuronal Per-Arnt-Sim domain protein 4 (Npas4). We also found that MAPK phosphorylated Npas4 downstream of PKA, increasing Npas4–CBP interaction and the transcriptional activity of Npas4 at the brain-derived neurotrophic factor (BDNF) promoter. Deletion of Npas4 in the D1R-expressing MSNs impaired cocaine-induced place preference, which was rescued by Npas4-WT but not by a phospho-deficient Npas4 mutant. These observations suggest that MAPK phosphorylates Npas4 in D1R-MSNs and increases its transcriptional activity to enhance reward-related learning and memory.

1-P-027

Piccolo knockdown in the perirhinal cortex induces cognitive dysfunction in the new schizophrenia mice

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Schizophrenia is a severe mental disorder with cognitive dysfunction as one of the core symptoms. Piccolo encoded by the *PCLO* gene serves at the nerve terminal, its knock down (KD) in mice prefrontal cortex (PFC) induced schizophrenia-like symptoms. The animal model shows cognitive dysfunction, however the mechanism has not been clarified. In this study, we investigated the role of the Piccolo in the perirhinal cortex (PRC) in cognitive function, since of bidirectional connections between the PFC and HIP via the PRC involved in memory consolidation.

AAV-miPCLO/GFP vectors was injected into the mice PRC (PRC-Piccolo-KD), and were assessed by behavioral and electrophysiological tests. PRC-Piccolo-KD mice showed memory impairments in the several tasks. Directional projection from PRC to ventral HIP was observed using GFP searching. LTP in the ventral HIP decreased in the PRC-Piccolo-KD mice.

Our findings suggest that Piccolo in the PRC plays an important role in synaptic plasticity in the ventral HIP via functional deficits of the PFC-PRC-HIP pathway, leading to schizophrenia-like cognitive dysfunction. Modulation of Piccolo in PRC will be a target for therapeutic approaches to schizophrenia-like cognitive dysfunction.

1-P-028

Establishment of cognitive dysfunction model mouse injected alpha synuclein in the brain

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It has been reported recently that there is a possibility that accumulation of α synuclein causes neurological problems and introduces central nerve system disorders. Most of the studies have been performed using genetically-modified animals or animals prepared with virus vectors. These animal models are not suitable for efficacy screening of drugs.

The present study was performed to establish an in vivo screening model which can be used easily for drug efficacy evaluation of cytotoxicity of α synuclein.

[Methods] An α synuclein was administered to the lateral ventricle of male mice at 6 weeks of age. Behavioral assessment was performed 7 to 12 days after administration by the Y-maze test and passive avoidance test.

[Results] The α synuclein solution administered to the lateral ventricle impaired short-term memories. The animals recovered from the impairment by donepezil administration.

[Compilation] A mouse model whose lateral ventricle was treated once with the α synuclein had impaired learning. This impairment was improved by donepezil. Therefore, the possibility is suggested that this mouse model can be an in vivo screening model which can be used easily in a short period for drug efficacy evaluation of cytotoxicity of α synuclein.

1-P-029

Age-dependent impairment of memory, neurofibrillary tangle formation and clearance in a mouse model of tauopathy

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Insoluble, fibrillar intraneuronal accumulations of the tau protein called neurofibrillary tangles (NFTs) are important hallmarks of the Alzheimer disease (AD) brain and play an important role in the behavioral phenotypes of AD. rTg4510 mice constitutively express mutant human tau until transgene expression is inactivated by administration of the doxycycline (DOX). The present study aimed to determine the initial phenotypic characterization of the rTg4510 mice and to define the relationship between the extent of memory deficit and the duration of NFTs overexpression. In 6-month-old (young) rTg4510 mice, both spatial memory and object recognition memory were impaired, and these impairments were recovered by pre-treatment with DOX for 2 months. In parallel, the expression of NFTs decreased in DOX treated group. 10-month-old (aged) rTg4510 mice showed strict impairment in memory performances and treatment with DOX could not recover these impairments. Increasing levels of NFTs were observed in aged rTg4510 mice. Treatment with DOX could not recover the tau pathology in aged rTg4510 mice as well as DOX-untreated group. These results suggest that clearance mechanisms of NFTs were impaired as of 10 months of age.

1-P-030

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-P-031

Tryptophan-tyrosine (WY) dipeptide improves memory impairment with aging via suppression of microglial inflammation

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Some epidemiological studies revealed that the consumption of dairy products prevents cognitive decline in the elderly. We previously demonstrated that tryptophan-tyrosine (WY)-peptide rich in Camembert cheese suppresses microglial inflammation. In the present study, 7-week-old (young) and 68-week-old (aged) C57BL/6J mice were fed diet containing WY-peptide. After 4 months of feeding, mice were subjected to behavioral evaluations of the Y-maze test and the novel object recognition test (NORT), which were decreased in aged mice compared to young mice, but those in aged mice with WY-peptide were improved. The levels of pro-inflammatory cytokines and glutamate in the hippocampus of aged mice were higher compared to young mice, while they were decreased by WY-peptide. These results suggest that WY-peptide consumptions suppress microglial inflammation in the hippocampus, resulting in memory improvement. Aged mice orally given WY-peptide or whey peptide rich in WY-peptide for shorter term (18 days) also improved the memory impairment. The present study demonstrated that long-term or short-term consumption of WY-peptide is beneficial for age-related memory impairment.

1-P-032

Effects of the extract of *Epimedium grandiflorum* on CNS and blood glucose level in adult male rats with hyperglycemia

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E. grandiflorum have a relatively weak inhibitor of PDE5 in comparison to substances like sildenafil (viagra). Furthermore, because of its estrogenic action and vasodilating action, it has been used as a therapeutic agent for vascular dementia. However, studies on the efficacy of *E. grandiflorum* as a drug for treating dementia are scarce. In this study, we investigated the effects of oral administrations of *E. grandiflorum* on the function of CNS and blood glucose level in adult male rats with hyperglycemia. We used a battery of behavioral tests including the Morris water maze test, an open-field test and the elevated plus-maze test. Rats were given normal food and water or high fat diet and 20% sucrose solution for 2 month, and they were put in a hyperglycemic state. After that, rats were orally administered with saline or *E. grandiflorum* extract (0.15 or 0.58 mg/kg/day) for 1 month from the beginning of 1 month after feeding high fat diet etc. It was shown that the extract of *E. grandiflorum* does not affect spatial learning memory, locomotor activity, emotionality and blood glucose level of hyperglycemic rats. It may need a higher amount of the *E. grandiflorum* extract to act as an anti-dementia drug.

1-P-033

Nucleic acids in hydrolyzed salmon milt extract promote proliferation of neural stem cells and enhance brain function in normal mice

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Salmon milt extract is known to improve impaired brain function in animal models with brain disease and contains high levels of nucleic acids. The purpose of the present study is to clarify the effect of hydrolyzed salmon milt extract (HSME) and its nucleic acid fraction (NAF) on brain function in normal mice. A diet containing 2.5% HSME or NAF, but not normal diet, induced normal mice to devote more time in exploring novel and moved objects than in exploring familiar and unmoved objects, as observed during novel object recognition and spatial recognition tests, respectively, suggesting that nucleic acids in HSME may enhance brain function. Quantitative PCR analysis revealed that expression of marker genes for neural stem cells (NSCs) and glial cells, followed by those of neurons, was up-regulated after start of the ingestion. Exposure of primary cultured NSCs to HSME, NAF, and oligonucleotides significantly increased MTT reduction activity and cellular ATP level, suggesting that nucleic acids directly promote proliferation in NSCs. Thus, oral ingestion of nucleic acids enhances brain function in normal mice, and this effect may be at least partially provoked by increase in proliferation of NSCs.

1-P-034

The involvement of EAAC1 in diurnal variation of ischemic Zn²⁺ 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 Zn²⁺ 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 Zn²⁺ 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-P-035

Expression of Arcadlin in the ischemic brain

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Previous studies suggest that the brain is capable of adapting to the brain injury due to ischemia, and that synaptic remodeling is important in this process. However, its biological mechanisms are not well understood. Arcadlin, a member of protocadherins, is involved in the synaptic plasticity, and *Arcadlin Knockout* increases the dendritic spine density *in vitro*. These notions imply the contribution of Arcadlin in the remodeling of neural network within the surviving tissue during the recovery from brain ischemia. In this study, we investigated the time course and the region of Arcadlin expression after cerebral ischemia. To induce permanent cerebral ischemia, C.B-17/Icr-+/+ Jcl wild-type male mice aged 7 weeks were subjected to middle cerebral artery occlusion (MCAO). Western blot analysis showed that Arcadlin is upregulated in hippocampus within 6 hours after MCAO. Sham operation did not induce the upregulation of Arcadlin. We would like to discuss about the possible involvement of Arcadlin during the pathophysiological processes after the cerebral ischemia.

1-P-036

Leukotriene B₄ secreted by microglia promotes neutrophil invasion into hematoma of mice with intracerebral hemorrhage

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Intracerebral hemorrhage (ICH) is devastating neurodegenerative disease that results from the leakage of blood constituents into brain parenchyma. Our previous research showed that leukotriene B₄ (LTB₄), a lipid mediator, promotes neutrophil invasion after ICH onset and exacerbates ICH pathology. Here we focused on microglia as the source of LTB₄ production. First, we checked the expression profiles of LTB₄ and other arachidonic acid metabolites in ICH brain. Male C57BL/6J mice were received the injection of typeVII collagenase (0.025 U) into striatum. Lipidomics analysis based on LC-MS/MS revealed the LTB₄ increase at 12 h after ICH induction. Then, we examined the effect of microglia-secreted mediators in ICH. Differentiated human promyelocytic leukemia cells (dHL-60) were seeded on upper layer of cell culture inserts with 3.0 µm pore, and treated culture supernatant of BV-2 cells with 12 h thrombin (30 U/mL) treatment to bottom layer. Culture supernatant of thrombin-treated BV-2 cells promotes infiltration of dHL-60 cells into the bottom layer, but U75302, a LTB₄ receptor 1 (BLT1) antagonist, suppressed the infiltration. These results suggest that LTB₄ secreted by microglia promote neutrophils invasion in ICH.

1-P-037

Effect of lipoxin A₄ for pathology of intracerebral hemorrhage mediated by microglial activation

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Intracerebral hemorrhage (ICH) results from the rupture of blood vessels and the leakage of blood constituents. Especially, thrombin, a blood coagulation factor, activates microglia and promotes ICH pathology. Furthermore, neutrophils infiltrated by blood leakage also exacerbates the ICH. Our previous study showed that leukotriene B₄ (LTB₄), a bioactive lipid, promotes pathological progression of ICH (Hijioka *et al.*, 2017). In this study, we focused on lipoxin A₄ (LXA₄), other bioactive lipid synthesized by the enzymes producing LTB₄. Thrombin (30 U/mL) treatment for 12 h increased mRNA expression of inducible nitric oxide synthase (iNOS) in BV-2 microglia. Then, differentiated human promyelocytic leukemia cells (dHL-60) induced by dimethyl sulfoxide were seeded on upper layer of cell culture inserts with 3.0 µm pore, and treated culture supernatant of BV-2 cells with 12 h thrombin treatment to bottom layer. Culture supernatant of thrombin-treated BV-2 cells promotes infiltration of dHL-60 cells into the bottom layer, but LXA₄ treatment suppressed the infiltration. These results suggest that mediators secreted by microglia promote neutrophils invasion and LXA₄ can be suppress the neutrophils invasion followed to ICH.

1-P-038

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-1 and 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-P-039

Development of human immortalized cell-based multicellular spheroidal blood-brain barrier model for CNS drug development studies

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Multicellular spheroidal (MCS) culture system is a promising culture method for development of a highly functional *in vitro* BBB model, which is expected to be a useful tool for central nervous system (CNS) drug development studies. By taking advantage of their scalability and functionality, immortalized cells are useful for development of such MCS-BBB models, and we have recently established human conditionally immortalized brain microvascular endothelial cells (HBMEC/ci18), astrocytes (HASTR/ci35), and pericytes (HBPC/ci37). Therefore, utilizing these cells, we aimed to develop human immortalized cell-based MCS-BBB models (hiMCS-BBB) and characterize their BBB properties. The cells in a V-bottom well were self-assembled into a spheroid, and HBMEC/ci18 formed the outer monolayer of the spheroids. Furthermore, while most HASTR/ci35 accumulated in the core, the majority of HBPC/ci37 aligned along the inner side of HBMEC/ci18. Next, we examined the BBB properties of hiMCS-BBB. We found that hiMCS-BBB showed 0.28-fold lower FITC-dextran (5 kDa) permeability compared with hiMCS-BBB without HBMEC/ci18. These results suggest that HBMEC/ci18 forms a functional barrier in hiMCS-BBB. Collectively, we have developed the hiMCS-BBB that exhibits BBB properties. The hiMCS-BBB holds the potential as a new *in vitro* experimental tool that will contribute to CNS drug development.

1-P-040

Effects of Sphingosine-1-phosphate on brain pericytes regulating blood-brain barrier functions.

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Blood-brain barrier (BBB) functions are maintained by cross-talk between brain capillary endothelial cells and elements of the neurovascular unit. Brain pericytes have crucial roles to maintain the BBB functions. Sphingosine-1-phosphate (S1P) is known as the regulator of many biological processes. The aim of the present study was to examine the role of S1P as interaction factors between endothelial cells and pericytes. To examine the effects of S1P on barrier functions, we made two kinds of in vitro BBB models, endothelial cells monolayer model and endothelium-pericytes co-cultured model. Barrier functions were assessed by measuring TEER and permeability of sodium fluorescein (NaF). S1P treatment decreased the TEER value and increased the NaF-permeability on endothelial monolayer model. The S1P-induced barrier dysfunctions were worsened by the presence of pericytes. To examine whether S1P affects the production of secreted factors from pericytes, real-time PCR was performed. S1P increased the several mRNA expression related to inflammation. These data indicate that secreted factors from pericytes stimulated by exogenous S1P worsen the BBB functions, and S1P act as the interplay factors in blood-brain barrier.

1-P-041

Behavioral characterization of APP knock-in mice model in touchscreen-based tests aiming early detection of Alzheimer's disease.

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Alzheimer's disease (AD) is a progressive disease characterized by loss of memory and other important mental functions. Accumulation of amyloid beta (A β) and fibrillary tangles are the main pathological hallmarks of this disease. Emerging data suggest that the disease process begins years before clinical diagnostic confirmation; thus, methods to improve early detection would provide opportunities for early intervention to delay progressive cognitive decline and disease onset. In our research we assessed the cognitive abilities of *APP*^{NL-G-F/NL-G-F} knock-in (*APP*-KI) mice with a touchscreen-based automated test battery and water maze test. These tests are mainly dependent on the brain regions that are prone to A β accumulation at the earliest stages of the disease. We subjected male 6- and 11-months old *APP*-KI mice in water maze test where only older mice showed significantly worsened behavior than wild-type mice. However, using touchscreen based behavioral test it was possible to detect cognitive impairment in *APP*-KI mice at an early (4 months) stage while classical behavioral tests shows comparable results between wild-type and disease model mice.

1-P-042

Involvement of SUMO1 in Alzheimer's disease pathology

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Small ubiquitin-like modifiers (SUMO) are covalently conjugated to target proteins and modulate a growing number of cellular pathways. SUMOs have been strong links to tumorigenesis and metastasis due to regulation of nuclear events. More recently, increasing evidence has demonstrated their role in neuronal pathways such as synaptic plasticity and memory as well as several neurodegenerative diseases.

In the current study, we examine the impact of SUMO1 on processing of the amyloid precursor protein (APP) leading to the production and deposition of the amyloid beta (A β) peptide. An in vivo model of these pathways was developed by the generation of double transgenic mice over-expressing human SUMO1 and a mutant APP. The SUMO1-APP transgenics exhibited increased insoluble A β and plaque density accompanied by increased dendritic spine loss, more pronounced synaptic and cognitive deficits at later ages. Then we examined several possible mechanisms for the SUMO1-mediated increase in amyloid load.

In this presentation, we will show our results and explain the possible involvement of SUMO1 in the pathology of AD.

1-P-043

Effects of gem-dihydroperoxides against mutant copper / zinc superoxide dismutase-mediated neurotoxicity

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that causes progressive loss of motor neuron. The mechanism of ALS involves the aggregation and accumulation of several mutant proteins, such as copper / zinc superoxide dismutase (SOD1). Previous reports have shown that excessive oxidative stress, associated with mitochondrial dysfunction and mutant protein accumulation, contributes to the progress of ALS. Having recently synthesized novel organic gem-dihydroperoxides (DHPs) with high anti-oxidant activity, we examined whether DHPs reduce the mutant SOD1-induced intracellular aggregates involved in oxidative stress. We found that, among DHPs, 12AC2O significantly inhibited mutant SOD1-induced cell death and reduced the intracellular mutant SOD1 aggregates. Moreover, immunofluorescence staining with redox-sensitive dyes showed that 12AC2O reduced the excessive level of intracellular mutant SOD1-induced reactive oxygen species (ROS). Additionally, ESR analysis showed that 12AC2O exerts a direct scavenging effect against the hydroxyl radical ($\cdot\text{OH}$) and the superoxide anion ($\text{O}_2\cdot^-$). Collectively, these results suggest that 12AC2O is a very useful agent in combination with other agents against ALS.

1-P-044

Tamibarotene increases PiT2 expression and has a potential maintaining inorganic phosphate homeostasis.

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Idiopathic basal ganglia calcification (IBGC) is an intractable disease characterized by bilateral calcification in basal ganglia and other regions. The most frequent causative gene of familial IBGC is *SLC20A2* which codes inorganic phosphate (Pi) transporter, PiT2. Then Pi homeostasis was thought to be disturbed in the brain of patients with *SLC20A2* mutations. This suggested that repairing Pi homeostasis in the brain led to the improvement of symptoms in patients with IBGC. Here, we aimed to screen drugs which stimulate PiT2 activity. The past study showed that some transcriptional factor bound promoter region of *SLC20A2*. Among them, Tamibarotene was proposed as a potential candidate by analyses of transcriptional factors-binding site in the *SLC20A2* promoter. We examined the effects of the candidate drugs using bEnd.3 cells. As a results, Tamibarotene significantly increased the levels of both *SLC20A2* mRNA and PiT2 protein. The amount of Pi uptake into cells was measured using radioisotope ³²P. The increases of Pi uptake was observed in the Tamibarotene-treated group. In conclusion, Tamibarotene increases PiT2 expression, suggesting a possibility improving Pi homeostasis.

1-P-045

Extraction of the active compound of Nrf2-ARE pathway derived from *Panax ginseng* C. A. Meyer

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Extraction of the active compound of Nrf2-ARE pathway derived from *Panax ginseng* C. A. Meyer
Oxidative stress induced by reactive oxygen species (ROS) generated in the human body is considered as a risk factor of various neurodegenerative diseases. Nrf2-ARE pathway is well-known as biological defensive system against the oxidative stress. In this study, we isolated and identified the Nrf2-ARE activator from *Panax ginseng* C. A. Meyer and then evaluated its actions against PC12 cells. First, the extract of *Panax ginseng* C. A. Meyer exhibited high ARE activity by luciferase reporter assay using PC12 cells. Dividing the extract into 9 fractions by reversed-phase HPLC, we determined the ARE activities of each fraction by reporter assay. Based on LC/TOF-MS, the active ingredient responsible for the ARE activity was identified as panaxytriol ((3R,9S,10S)-Heptadec-1-en-4,6-diyne-3,9,10-triol). Importantly, the pretreatment of panaxytriol significantly protected native PC12 cells from 6-OHDA toxicity. Additionally, NQO1 assay revealed that panaxytriol raised NQO1 activity in native PC12 cells. These results suggest that the extract of *Panax ginseng* C. A. Meyer contains panaxytriol as a Nrf2-ARE activator and has the cytoprotective effect against oxidative stress by inducing some antioxidant enzymes.

1-P-046

Effect of glucocerebrosidase inhibition on the activities of microautophagy and chaperone-mediated autophagy

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Glucocerebrosidase (GCase) is localized in lysosome and degrades glucocerebroside into glucose and ceramide. It has been reported that a decrease in GCase activity by mutations is one of the risk factors of Parkinson's disease (PD) via the accumulation of α -synuclein. In addition, the accumulation of α -synuclein impairs protein degradation via autophagy-lysosomal pathway (ALP). ALP is classified into macroautophagy (MA), microautophagy (mA) and chaperone-mediated autophagy (CMA). Recent reports revealed that Hsc70, a mA- and CMA-related protein, is decreased in PD patients, suggesting the involvement of mA and CMA in PD pathogenesis. We have established a novel method to assess mA and CMA activities in cultured cells. In the present study, we investigated whether the inhibition of GCase affects mA/CMA activity using this method. Conduritol- β -epoxide (C β E), a GCase inhibitor, did not affect mA/CMA activity in AD293 cells. However, C β E significantly decreased it in cells transfected with α -synuclein. These findings suggest that the inhibition of GCase impairs mA/CMA activity in the presence of α -synuclein, leading to further accumulation of α -synuclein. The impairment of mA/CMA would be related to PD pathogenesis.

1-P-047

Plasmalemmal voltage-dependent anion channel is one of membrane proteins targeted for 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂

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Voltage-dependent anion channel (VDAC) has a crucial role in apoptosis. VDACs are not only expressed in mitochondria but also on plasma membrane. In the present study, we confirmed that VDAC was detected in the neuronal plasma membrane and localized on the neuronal cell surface. By proteomic approach, VDAC was identified as one of membrane targets for 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂ (15d-PGJ₂), which activated caspase and induced neuronal cell death. The bound of VDAC with 15d-PGJ₂ was detected by pull-down assay. VDAC was partially co-localized with membrane targets of 15d-PGJ₂, suggesting the partial involvement of VDAC in the neurotoxicity of 15d-PGJ₂. To target only plasmalemmal VDAC, but not mitochondrial one, the anti-VDAC antibody was applied without permeabilization. The anti-VDAC antibody significantly attenuated the neurotoxicity of 15d-PGJ₂, but not completely. The antibody significantly reduced the 15d-PGJ₂-activated caspase 3. Thus, we suggested that plasmalemmal VDAC might contribute partially to the neurotoxicity of 15d-PGJ₂ as one of membrane targets.

1-P-048

***Xenopus slc7a5* is essential for notochord function and primary neurogenesis via sonic hedgehog signaling**

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SLC7A5 (also known as LAT1), largely accepted as an amino acid transporter, has been shown to play important roles in cancer and developmental process. Because knockout mice of *slc7a5* are embryonically lethal due to placental defects, it is difficult to evaluate its role in early development. In this study, *slc7a5* expression and function was evaluated in *Xenopus laevis* embryos that do not require the placenta. Expression of *slc7a5* was detected in the notochord and in the eye from gastrula stage and it was not co-localized with *slc3a2*, which helps *slc7a5* to localize at the plasma membrane, before late neurula stage. Loss-of-function experiment with morpholino antisense oligonucleotide led to early neural patterning defect and inhibition of primary neurogenesis. These defects were likely due to impaired notochord development as sonic hedgehog (shh) signaling pathway was compromised in *slc7a5*-inhibited embryos. These results suggest that *slc7a5* is required for notochord development and subsequent neural patterning via shh/gli signaling. In early stages of neural development, the function of *slc7a5* appeared to be independent of transport function.

1-P-049

Effects of coriander (*Coriandrum sativum* L.) on GABA neuron in mouse brain

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Coriander is taken up as a "new food materials" in Japan, although this has been used as folk remedies for Ebers papyrus, an ancient medical record in Egypt over 3000 years. And many reports documented that coriander has not central functions. In this study, we search for the mechanism of the central effects of coriander using a behavioral study and the RT-PCR. **Method:** ① ICR mice (male:6 weeks old) were used as experimental animals. Animal groups were divided into 4 groups such as water group and 3 groups of coriander (100, 200, 400mg/kg). ② Stages of sedation was controlled by the dosage of pentobarbital. ③ Central excitatory effects were indexed by pentetrazol (PTZ) convulsion. ④ Spontaneous activities of mice were measured at night. ⑤ Changes in GABA_A receptor, NMDA receptor, GABA transporter expressions were measured by RT-PCR. **Results:** ① A significant sedative effect was observed in the coriander group. However, there was no significant difference in diurnal activities. ② As a result of PCR, the suppression of GABA uptake in the brain was detected under PTZ administration in the coriander group. **Conclusion:** These results suggest that long-term continuous oral administration of coriander can cause the mild sedation due to the GABA neuronal system.

1-P-050

Seizure susceptibility of *Phf24*-knockout rats

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Phf24 interacts with G_{ai} subunit and facilitates GABA_B receptor functions. We previously demonstrated that Phf24 expression was markedly down-regulated in Noda epileptic rat (NER) (Behav. Genet., 47, 609, 2017). To clarify the role of Phf24 in regulating seizure susceptibility, we created the novel animal model, *Phf24*-knockout (KO) rats, and analyzed their behavioral phenotypes. Seizure susceptibility of *Phf24*-KO rats was assessed using chemically- and electrically-induced seizure tests. Behavioral score and incidence of seizures induced by pentylenetetrazole (PTZ, 30-40 mg/kg) and pilocarpine (300 mg/kg) were significantly increased in *Phf24*-KO rats than in control (F344) rats. *Phf24*-KO rats also showed higher sensitivity to electrical shock-induced seizures. In addition, PTZ-induced kindling (30 mg/kg/day, 10 days) was significantly facilitated by the *Phf24*-KO. Furthermore, immunohistochemical analysis of c-Fos expression, a biological marker of neural excitation, revealed that *Phf24*-KO rats showed a significantly higher Fos expression than control animals in the cerebral cortex, amygdala, hippocampus and thalamus. These results suggest that Phf24 play a crucial role in controlling the susceptibility to epileptic seizures, which is probably involved in epileptogenicity of NER.

1-P-051

The mechanism underlying the propofol-induced elevation of intracellular calcium

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Propofol, most frequently used as general anesthetic, is thought to exert its anesthetic actions via GABA_A receptors, however the precise mechanisms of its adverse actions remains unclear. We examined the propofol-induced elevation of intracellular calcium using SHSY-5Y neuroblastoma cells loaded by calcium indicator Fluo-4. Propofol at 0 – 200 μM elevated the intracellular calcium in a dose-dependent manner. This phenomenon was not influenced by the elimination of extracellular calcium. And, the calcium elevation was abolished when intracellular or intra-endoplasmic reticulum (ER) calcium was depleted by BAPTA-AM or thapsigargin, respectively, suggesting that the calcium was mobilized from ER. Studies using various inhibitors including U-73122, Xestospongin C and dantrolene revealed that the propofol-induced calcium elevation was not mediated through G-protein coupled receptors, IP3 receptors or ryanodine receptors. We captured the live imaging of ER during the propofol stimulation using ER-tracker. Accompanying the calcium elevation, the ER structure was fragmented and aggregated, which was gradually restored. These phenomena might be involved in the exertion of various adverse effects of propofol including angialgia and propofol infusion syndrome.

1-P-052

Cardiac myofibroblasts engulf dead cells generated in hearts with hypertrophy

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Cardiac hypertrophy is one of the major pathogeneses of heart failure. In cardiac hypertrophy, dead cardiomyocytes are supposed to be promptly engulfed by phagocytic cells. However, the cells and molecules responsible for removing dead cardiomyocytes in cardiac hypertrophy have not yet been revealed.

We performed transverse aortic constriction (TAC) operation on wild-type (WT) mice to generate mouse models of cardiac hypertrophy. Among the several molecules mediating engulfment, the expression level of MFG-E8, a molecule that promotes engulfment, in the heart was significantly increased after TAC operation. MFG-E8 was specifically expressed in myofibroblasts. We revealed that myofibroblasts have the ability to engulf dead cells via MFG-E8. Consistent with these results, the cardiac conditions of TAC-operated MFG-E8 KO mice were markedly worse compared with those of WT mice. In addition, exogenous expression of MFG-E8 in hearts of WT mice by the adeno-associated virus significantly improved the cardiac condition after TAC operation. In conclusion, we identified cardiac myofibroblasts and MFG-E8 are responsible for the removal of dead cardiomyocytes in cardiac hypertrophy.

1-P-053

Involvement of RanBPM in HDAC6-mediated regulation of microtubule stability after myocardial infarction in rats

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When chaperone and proteasome systems are impaired, ubiquitinated proteins are transported along microtubules to aggresomes. Histone deacetylase 6 (HDAC6) is a key factor for aggresome formation, since it has ubiquitinated protein and dynein motor binding domains. In addition, HDAC6 deacetylates α -tubulin and regulates microtubule stability. In this study, we examined changes in the acetylation level of α -tubulin by HDAC6 in the development of heart failure (HF) after myocardial infarction (MI). MI was induced by coronary artery ligation (CAL). Hemodynamic parameters at the 8th (8W), but not 2nd (2W), week after CAL showed signs of HF. Myocardial HDAC6 and acetylated α -tubulin contents were increased in 2W-CAL rats, whereas those in 8W-CAL rats were similar to the corresponding Sham rats. These findings indicate that changes in HDAC6 content are not consistent with the acetylation level of α -tubulin after CAL. We focused on Ran-binding protein M (RanBPM), which interacts with HDAC6 to suppress its activity. Changes in content of RanBPM after CAL were similar to those of HDAC6. These results suggest that RanBPM enhances microtubule stability through a suppression of HDAC6 activity and promotes aggresome formation.

1-P-054

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

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An impairment of mitochondrial energy-producing ability leads to the development of heart failure (HF) following myocardial infarction (MI). In this study, effects of cardiac progenitor cell (CPC) transplantation to the cardiac tissue after MI on the cardiac mitochondrial energy-producing ability were examined. MI was produced by ligation of the left ventricular coronary artery. Immediately after MI, Sca-1⁺, c-Kit⁺, or crude CPCs were injected into viable myocardium. Eight weeks after MI, animals without transplantation showed typical signs of HF. The mitochondrial oxygen consumption rate (mtOCR) of the viable tissue in rats with HF was reduced. In contrast, the cardiac function and mtOCR were preserved in CPC-transplanted groups. Furthermore, mtOCR in the rats with transplantation of crude CPC was slightly higher than other groups. These results suggest that the transplantation of CPCs contributes to a preservation of mitochondrial function, leading to an improvement of cardiac function. We also found that there were differences in cardioprotective effects among Sca-1⁺, c-Kit⁺, and crude CPCs.

1-P-055

Voluntary wheel running may improve cardiac dysfunction in experimental mouse model of cancer-induced cachexia

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A rat model of cancer cachexia has recently been established by implantation of the human stomach cancer cell line, which shows similar symptoms observed in human patients. On the other hand, cardiovascular diseases in cancer patients with cachexia have become a great concern. Here we applied this cancer cachexia model to mice and evaluated symptoms of cachexia including cardiac functions. Moreover, we investigated effects of voluntary wheel running (VWR) on cachexia symptoms using this model.

85As2 human stomach cancer cells were inoculated to male BALB/c nu/nu mice, which showed a symptomatic cachexia at 2 wks after cancer implantation. By 8 wks after implantation, severe cardiac atrophy was developed and left ventricular ejection fraction (LVEF) was markedly reduced. VWR starting from 2 to 6 wks after implantation significantly suppressed the severity of cachexia. Moreover, LVEF significantly increased in cachexia group with VWR, compared to cachexia group without VWR.

In our cachexia mouse model, voluntary exercise could improve cachexia-induced cardiac dysfunction as well as suppress a progress of cachexia itself, suggesting a possible therapeutic effect of exercise on heart failure induced by cancer cachexia.

1-P-056

Evaluation of cardiac function in a knock-in mouse model for human DCM at early postnatal stages

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Purpose: Dilated cardiomyopathy (DCM) is a most common cause of cardiac transplantation in children. However, little is known about the disease progression process of DCM in children. In this study, we explored the disease progression of DCM during early postnatal stages, using a knock-in mouse model for human DCM caused by $\Delta K210$ mutation in the cardiac troponin T gene.

Methods: Cardiac functions were evaluated at 15 and 30 days old, using M-mode echocardiography and color Doppler.

Results: BW of mice at 30 days old was about 2-fold greater than at 15 days old. At 15 days old, HW of HM mice were already greater than WT. At 30 days old, HW of heterozygous (HT) mice also became greater than WT. LVIDd of DCM mice was significantly greater than WT both at 15 and 30 days old. LV wall thickness was not different between DCM and WT mice at 15 days old, but thinner in DCM mice at 30 days old. EF and FS declined in HM but maintained in HT at both ages. Fibrosis was observed only in 30 days old HM mice.

Conclusions: LV dilation and systolic dysfunction occur at a very early postnatal stage before weaning in this DCM mouse model, suggesting that this model is useful for the exploration of pathogenic mechanisms and therapeutics for very early onset DCM.

1-P-057

Daily voluntary exercise suppresses progression of heart failure in DCM model mice.

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Today, exercise is regarded as one of therapies for heart failure (HF). However, the effects of exercise on patients with dilated cardiomyopathy (DCM) have not been established. A knock-in mouse model of human inherited DCM, *TNNT2* Δ K210, shows similar characteristics to DCM patients. We aimed to examine how the frequency of voluntary exercise influence progression of heart failure using the DCM model mice. Homozygous Δ K210 (DCM) mice showed enlarged heart and frequent sudden death with $t_{1/2}$ of ~ 70 days. DCM mice were divided into 3 groups based on the frequency of voluntary exercise: no exercise control, every 2 days (2D) and daily exercise (ED). The 2D and ED groups started running at 1 month of age. At the 2 months of age, mice were sacrificed after an investigation with echocardiography, and their heart, lung, lower extremity muscles and body weights were measured. Gene expressions of HF- and arrhythmia-related genes in myocardium were quantified by qPCR analysis. The ejection fraction was significantly improved in ED group compared with 2D and control. ED group showed attenuated electrical remodeling in the hearts. These result indicated that daily voluntary exercise prevents progression of HF in DCM mice.

1-P-058

Identification of differentially expressed genes in post-cardiac arrest syndrome treated with H₂ inhalation in rats: A DNA microarray study

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[Background] Our previous data demonstrated that hydrogen (H₂) inhalation improved cardiac function in a rat model of cardiac arrest. In this study, we examined the genes expression of post-cardiac arrest treated with H₂ using a DNA microarray based comprehensive approach. [Methods and results] Rats were subjected to 6 minutes of ventricular fibrillation cardiac arrest followed by cardiopulmonary resuscitation. Resuscitated rats were mechanically ventilated with 26% O₂ with or without 1.3% H₂. Sham-operated rats had the same operative procedure without cardiac arrest. Animal survival rate on day 7 was 38.4% (control group) vs. 71.4% (H₂ treated group). We isolated hearts on 7 days after resuscitation, and then analyzed gene expression using a DNA microarray. One hundred fifty-one genes were up-regulated while 109 were down-regulated genes in cardiac arrest rats. In addition, 511 genes were increased and 461 genes were decreased in H₂ treated group than those of the untreated group. Hierarchical clustering algorithm also showed obvious differences in comprehensive gene expressions between the untreated and H₂ treated group. [Conclusion] Our data demonstrated that H₂ inhalation on post-cardiac arrest syndrome.

1-P-059

Preventive effect of olmesartan on right ventricular fibrosis in rats with monocrotaline-induced pulmonary hypertension.

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Heart failure caused by right ventricular hypertrophy and the fibrosis is a major cause of death in pulmonary hypertension. It has been reported that certain angiotensin AT₁ receptor blocker possesses inhibitory effect on cardiovascular remodeling. Thus, in the present study, we investigated whether olmesartan, an angiotensin AT₁ receptor blocker, could attenuate right ventricular hypertrophy and/or fibrosis using rats with monocrotaline-induced pulmonary hypertension.

Male Sprague-Dawley rats (5-week-old) were administered single subcutaneous injection of monocrotaline (60 mg/kg) and induced pulmonary hypertension. Olmesartan continuously infused subcutaneously for 4 weeks by use of an osmotic mini pump. The estimated dosage of olmesartan during the experimental period was around 3 mg/kg/day.

Treatment with olmesartan failed to improve right ventricular hypertrophy, whereas it reduced right ventricular fibrosis in the monocrotaline-treated rats. Moreover, enhanced expression of fibrosis-related proteins including IL-6, IL-1 β , GDF15, CTGF, and MMP9 the rats was not observed in the olmesartan-treated rat right ventricles. These results suggest that olmesartan has a potential to be a therapeutic agent against pulmonary hypertension.

1-P-060

Eucommia ulmoides oliver leaf extract improves the development of hypoxia-induced pulmonary arterial hypertension.

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Eucommia ulmoides Oliver is known as an herbal medicine. Recently, *Eucommia ulmoides* oliver leaf extract (ELE) has been shown to improve vascular function and vascular hypertrophy in SHR. Pulmonary arterial hypertension (PAH) is a severe and progressive disease that causes right heart failure. The pathogenesis of PAH is generally characterized by persistent high pulmonary arterial resistance and pulmonary arterial remodeling. In the present study, we investigated the effects of ELE on hypoxia-induced PAH in mice. 10-weeks-old male C57BL/6J mice were orally administered a 5% ELE during exposure to hypoxia for 4 weeks. ELE significantly suppressed the elevation of right ventricular systolic pressure in hypoxia-induced PAH mice. In addition, hypoxia-induced pulmonary arterial muscularization was tended to be attenuated in ELE-treated mice. Our findings suggest that ELE may effectively improve the development of hypoxia-induced PAH by preventing the hypercontraction and/or vascular remodeling of pulmonary artery.

1-P-061

Oroxylin A enhances rat serum-induced contractions via activation of the 5-HT/GRK2 pathway in smooth muscle cells of rat tail arteries

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Due to the limited treatment options for refractory hypotension and vascular hyporeactivity to vasoconstrictors, the new drug development is needed. The effect of the naturally-occurring flavonoids, oroxylin-A, wogonin and baicalein, in the presence of serum on the tensions of rat tail arteries was examined by using *in vitro* blood-vessel myography. We found that oroxylin-A (1-300 μ M) dissolved in rat serum (OroA/RS), promoted RS-induced but endothelium-independent vasoconstrictions in a concentration-dependent manner. On the other hand, baicalein and wogonin (structurally close to OroA) did not affect or attenuated RS-induced constrictions in tail arterial rings. Repeated applications of OroA (300 μ M)/RS induced reproducible and long-lasting constrictions without tachyphylaxis in the arterial rings. OroA/RS-induced vasoconstrictions were blocked by 5-HT_{2A} receptor antagonist (ketanserin, 0.3 μ M) and GRK2 antagonist (CMPD101, 10 μ M), but not by the inhibitors of ET-1 receptor, AT-1 receptor, or EP1/2 receptor. These results suggest that OroA with its special structure may potentiate the endothelium-independent contractions induced by RS via the 5-HT/GRK2 pathway in tail arteries. The intervention of OroA may be beneficial for the acute management of endotoxemic hypotensive shock.

1-P-062

The effect of quercetin on aortic aneurysms and dissection in pharmacologically-induced model mice.

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Purpose: Aortic aneurysm (AA) and aortic dissection (AD) are common diseases among the elderly people and potentially lethal. However, there is currently no management to prevent these diseases. Quercetin, an abundant polyphenol in onions, is reported to improve vascular function. In this study, we investigated the effects of quercetin on AAs or AD in mice. Methods: In C57BL/6J male mice, angiotensin II (Ang II) and β -aminopropionitrile (BAPN) were administered to induce hypertension and degeneration of elastic lamina respectively, leading to AAs. If L-NAME were added, mice shows endothelial dysfunction and more AD incidence. We call these AA model as "AB" and AD model as "LAB". Results: Quercetin treatment reduced the incidence of AAs and the death from aortic rupture in AB mice, and the incidence of AD and rupture in LAB mice. The activity of MMP-2/9 were upregulated in aortae, but suppressed by quercetin treatment. The expressions of vascular endothelial cell adhesion molecule-1 and F4/80, a marker of macrophages, are also suppressed in quercetin treated group. Conclusions: These findings suggest that quercetin prevents AA and AD via its protective effects against endothelial dysfunction, elastin degeneration and inflammation.

1-P-063

Involvement of latent TGF-beta binding protein 4 (LTBP-4) and Fibulin-5 in age-associated changes in aortic morphology and function

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Extracellular matrix (ECM), mainly composed of elastin and collagens, is a fundamental structural component participating in vascular function. Previously, our group demonstrated that LTBP-4 and Fibulin-5 are key molecules in regulating elastic fiber formation. Here, we analysed quantitative changes of LTBP-4 and Fibulin-5 with aging in mice. Thoracic aortas were resected from wild-type (WT) mice from two- to 48-weeks of age and *Ltbp4S*-ko or *Fbln5*-ko mice at four-months after birth. Expression of LTBP-4 or Fibulin-5 was evaluated by Western blot. Three-dimensional structures of elastin and collagen were visualized using two-photon microscopy. In WT mice, LTBP-4- and Fibulin-5 protein levels in aorta decreased with aging. Compared to young WT mice, higher degree of elastin break and collagen accumulation were found in ascending aortic wall of old WT, *Ltbp4*- and *Fbln5*-ko mice, suggesting a similar pathophysiology of increased aortic stiffness in these mice. In accordance, pulse pressure in old WT- and ko mice was higher than that in WT young mice. Further, higher incidence of aortic disease (aneurysm or dissection) induced by angiotensin II infusion was found in *Fbln5*-ko than WT mice. Thus, ECM remodeling caused by progressive decrease in intrinsic LTBP-4 and Fibulin-5 levels may induce age-related aortic stiffening and vascular disease such as dissection and aneurysm.

1-P-064

Matrix Gla protein negatively regulates calcification of human aortic valve interstitial cells isolated from calcified aortic valves

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Aortic valve stenosis (AS) is a common heart valve disease in elderly people, and is mostly accompanied by ectopic valve calcification. We recently demonstrated that tumor necrosis factor- α (TNF- α) induces calcification of human aortic valve interstitial cells (HAVICs) obtained from AS patients. In this study, we investigated the role of matrix Gla protein (MGP), a known calcification inhibitor that antagonizes bone morphogenetic protein 2 (BMP2) in TNF- α -induced calcification of HAVICs. HAVICs isolated from aortic valves were cultured, and calcification was induced with 30 ng/mL TNF- α . Gene expression of the calcigenic marker, BMP2, was significantly increased in response to TNF- α , while the gene and protein expression of MGP was strongly decreased. To confirm the role of MGP, MGP-knockdown HAVICs and HAVICs overexpressing MGP were generated. In HAVICs, in which MGP expression was inhibited by small interfering RNA, calcification and BMP2 gene expression were induced following long-term culture for 32 days. In contrast, HAVICs overexpressing MGP had significantly decreased TNF- α -induced calcification. These results suggest that MGP acts as a negative regulator of HAVIC calcification.

1-P-065

iNOS protects vascular smooth muscle cells from cell death stimulated by cyclic mechanical stretch via the p38 signal pathway.

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Cyclic mechanical stretch (CMS) leads to vascular smooth muscle cells proliferation, cell death and migration, resulting in vascular remodeling and subsequent vascular failure. However, the effect of CMS on gene induction in cardiovascular diseases remains to be determined. We have revealed that CMS caused cell death in rat aortic smooth muscle cells (RASMCs) in JNK and p38-dependent manners. To explore the causal role of CMS in initiating cell death signaling and MAPKs events, we compared transcript profiles of CMS-induced RASMCs death using cDNA microarrays. Inducible nitric oxide synthase (iNOS) gene was identified as having significantly differential expression in response to CMS. We further identified using qPCR analysis that CMS induced iNOS expression in a p38-dependent manner in RASMCs. The result also showed that NO production was increased, implying that NO was synthesized by CMS-induced iNOS. In contrast, NO production was inhibited by p38 inhibitor. Moreover, a iNOS inhibitor strongly increased CMS-induced cell death; whereas a NO donor significantly inhibited CMS-induced cell death in RASMCs, indicating that iNOS protects RASMCs from CMS-stimulated cell death via the p38 signal pathway.

1-P-066

Vascular Soluble Guanylate Cyclase Redox State in Rats with Chronic Administration of Cigarette Smoke Extract

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Smoking is known to be accompanied with a decrease in nitric oxide (NO) bioavailability in the vascular system. This study investigated whether chronic administration of cigarette smoke extract (CSE) influences on soluble guanylate cyclase (sGC) redox state, a determinant of NO bioavailability. Rats were subcutaneously administered phosphate saline buffer (PBS), gas phase-CSE (gp-CSE) or whole phase-CSE (wp-CSE) for 4 weeks, and vascular reactivity was examined in organ chamber experiments. In both the aorta and pulmonary artery, the relaxant response to acetylcholine was attenuated to a similar extent by administration of gp-CSE or wp-CSE. On the other hand, regardless of vessel type, sodium nitroprusside (reduced sGC stimulant)-induced and BAY 60-2770 (oxidized/heme-free sGC stimulant)-induced relaxation were identical in the three groups. These findings suggest that chronic CSE administration induces endothelial dysfunction but has no significant impact on vascular sGC redox state.

1-P-067

The effect of M-1, a major metabolite of sarpogrelate, on 5-hydroxytryptamine-mediated vasoconstriction in isolated human internal thoracic artery

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M-1 is a major metabolite of sarpogrelate, a selective inhibitor of 5-hydroxytryptamine (5-HT)_{2A} receptor. Our aim was to evaluate the effect of M-1 on the 5-HT-induced vasoconstriction in isolated endothelium denuded human internal thoracic artery (ITA) obtained from patients undergoing coronary bypass surgery. We investigated the effects of M-1, sarpogrelate and SB224289, a selective antagonist of the 5-HT_{1B} receptor, on the 5-HT-induced vasoconstriction. The vasoconstriction induced by 5-HT was significantly inhibited by M-1 in a concentration-dependent manner. Supramaximum concentrations of sarpogrelate or SB224289 significantly, but not completely, inhibited the 5-HT-induced vasoconstriction. In addition, simultaneous pretreatment with both sarpogrelate and SB224289 almost completely inhibited the 5-HT-induced vasoconstriction. M-1 also significantly almost completely inhibited the 5-HT-induced vasoconstriction, which mimics the effects of the simultaneous pretreatment with sarpogrelate and SB224289. These results demonstrate that M-1 inhibits 5-HT-induced vasoconstriction via the blocking activity of not only 5-HT_{2A} receptors but also 5-HT_{1B} receptors in human ITA.

1-P-068

Functional analyses of magnesium transporters using several genetically altered mice

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Magnesium ion (Mg^{2+}) plays an essential role in various cellular functions. Mg^{2+} deficiency or abnormal Mg^{2+} metabolism is related to various cardiovascular diseases, such as ischemic heart disease and arrhythmias. Recently, various candidate genes of Mg^{2+} transporters are reported, but their functional roles are still unknown. We first treated mice with three kinds of magnesium diets (low-magnesium diet, normal-magnesium diet, or high-magnesium diet) for 4 weeks, and found that the tissue expression levels of several Mg^{2+} transporters were dependent on magnesium intake. We also found that phenylephrine-induced contraction was attenuated in isolated aorta from low-magnesium-fed mice. Furthermore, to investigate the functional roles of these Mg^{2+} transporters, we generated several genetically altered mice targeting their Mg^{2+} transporters. Interestingly, phenylephrine-induced contraction was reduced in isolated aorta from these genetically altered mice. On the other hand, when these genetically altered mice were fed with a high-magnesium diet, the phenylephrine-induced contraction was recovered to normal level. These results suggest that these Mg^{2+} transporters may play important roles in the maintenance of Mg^{2+} homeostasis and vascular functions.

1-P-069

Cardiovascular functions in transgenic mice overexpressing dominant negative TRPM7 mutant

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Magnesium ion (Mg^{2+}) is an essential divalent cation, and intracellular Mg^{2+} concentration is tightly controlled by various Mg^{2+} transporters. Therefore, Mg^{2+} transporter dysfunction may consequently lead to a variety of diseases, such as cardiovascular, neuronal, and muscular diseases. Recently, several candidate genes for Mg^{2+} transporters have been identified. However, the regulation mechanisms of Mg^{2+} homeostasis are mostly unknown. To clarify these issues, we focused on Mg^{2+} -permeable non-selective cation channel TRPM7, and generated kidney-specific transgenic mouse model overexpressing the dominant negative TRPM7 mutant (M7DN-Tg), as an experimental tool. We confirmed that TRPM7 currents in HEK293 cells were almost completely inhibited by co-expression of the M7DN construct. We found that M7DN-Tg exhibited dysregulation of serum Mg^{2+} level and urinary Mg^{2+} excretion. Interestingly, vascular contractile responses in M7DN-Tg were significantly attenuated compared to the responses in wild-type mice. In M7DN-Tg, Mg^{2+} -enriched diet recovered the abnormal responses to the normal level. These results suggest that TRPM7 is involved in the regulation of Mg^{2+} homeostasis. M7DN-Tg will be a useful animal model for studying magnesium disorders.

1-P-070

Molecular mechanism underlying regulation of vascular smooth muscle phenotype switching by TRPC6

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Vascular smooth muscle cells (VSMCs) play critical roles in vascular homeostasis regarding stability and tonic regulation. VSMCs can switch their phenotype back and forth between highly proliferative synthetic and fully differentiated contractile in response to changes of vessel environment. Although critical for vascular homeostasis, this so-called phenotype switching is a cause of vascular diseases such as atherosclerosis and hypertension. In pathological conditions, proliferation of VSMCs are accelerated, which adversely affects prognosis. Therefore, the mechanism underlying transition from active synthetic to quiescent contractile phenotype has attracted attention but is largely unknown. In this study, we investigated the importance of canonical transient receptor potential 6 (TRPC6) in VSMCs phenotype switching. TRPC6 deficient (TRPC6(-/-)) VSMCs was more sensitive to the differentiation stimuli. We revealed that TRPC6(-/-) VSMCs have more polarized membrane potential and higher Akt activity than wild type cells under the differentiation pressure. TRPC6 physically and functionally coupled with lipid phosphatase PTEN, a negative regulator of Akt activation. These findings indicate suppression of TRPC6 can facilitate VSMCs differentiation and novel therapeutic strategy for several vascular diseases.

1-P-071

Hesperidin improves vascular remodeling in cuff-induced vascular injury mouse model

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Recently, we reported that drinking of *Citrus unshiu* juice (CU) or *Citrus iyo* juice (CI) drinking attenuated vascular remodeling in vascular injury mouse model. This effect was more marked with CI. Therefore, we focused on flavanone, hesperidin, which is more abundantly contained in CI compared with CU, and investigated the effect of hesperidin in vascular injury in mice. Eight-week-old male C57BL/6 mice were administrated 100 mg/kg/day hesperidin orally by gavage. Two weeks after administration, vascular injury was induced by polyethylene cuff placement on the femoral artery. Neointima formation was determined 14 days after cuff placement by evaluating intima/media ratio. One week after cuff placement, mRNA levels were measured by quantitative real-time RT-PCR. Treatment with hesperidin did not change systolic blood pressure and body weight compared with that in control mice. Neointima formation in the injured artery was significantly increased 2 weeks after cuff placement. Treatment with hesperidin significantly decreased neointimal formation. Expression of mRNA of tumor necrosis factor (TNF)-alpha and monocyte chemoattractant protein (MCP)-1 were increased by cuff placement. These increases tended to decrease in treatment with hesperidin. These results suggest that the intake of hesperidin in citrus fruits juice should prevent vascular injury.

1-P-072

Effects of 12 compounds on field potential signals with xCELLigence CardioECR system using iCell cardiomyocyte²

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Human iPSC cardiomyocytes (hiPS-CMs) have been used for the risk assessment of drug-induced QT prolongation and ventricular tachycardia called torsade de pointes. We have evaluated 12 compounds using CardioECR. The 12 compounds include high (azimilide, bepridil, dofetilide, ibutilide), intermediate (chlorpromazine, cisapride, clarithromycin, clozapine) and low (diltiazem, loratidine, metoprolol, mexiletine) risk classes. The effects of these compounds on field potential (FP) and impedance signals were evaluated with iCell cardiomyocytes². In the high risk group, azimilide, dofetilide and ibutilide prolonged the field potential duration (FPD) and induced EADs, but bepridil stopped the beating at the highest concentration. In low risk group compounds, diltiazem, metoprolol and mexiletine stopped beating, and loratidine showed no apparent change in FPD. The intermediate compounds stopped beating or induced EADs at higher concentrations. These results suggested that CardioECR can be used as a platform to assess the QT risk with hiPS-CMs. As CardioECR can utilize the impedance data in addition to the FP signals, an integrated analysis using both signals is useful for more accurate interpretation of compound nature.

1-P-073

Combined analysis of drug effects by impedance signals and live cell imaging obtained in the same iPSC cardiomyocytes preparation

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Although impedance has been increasingly used to detect the proarrhythmic and contractile abnormalities in iPSC cardiomyocytes (CM), there are still ambiguities in interpreting impedance signals to explore the mechanisms of drug actions. In the present study, we have measured the impedance signals with xCELLigence CardioECR and sequential live imaging with CQ1 to examine the correlation of impedance with Ca²⁺ transients, action potentials, and muscle motion in the same preparation of iCell CM². Beat duration of impedance signals showed the frequency dependent nature as with those of action potential, and had good correlation with Ca²⁺ signals in the change of signal duration induced by CiPA II non-core site compounds. Early afterdepolarization induced by cisapride could be identified more clearly in the Ca²⁺ or action potential signals than impedance ones. The peak amplitude was reduced by the calcium channel blockade in any of impedance, Ca²⁺ and motion signals, but the correlation among these signals was not likely to be clear compared with those of duration parameters. The combination of impedance signals and imaging data can be a strong tool to elucidate mechanisms of drug action on iPSC CM.

1-P-074

Electropharmacological effects of multi-ion channel blockers assessed in hiPSC-CMs sheets with MEA system

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[Purpose] We examined electrophysiological indices of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) sheets in order to quantitatively estimate multichannel blocking actions of bepridil and amiodarone using MEA system in comparison with that of E-4031. [Methods] We analyzed the field potential duration(FPD), effective refractory period, current threshold and conduction property using a programmed electrical stimulation protocol to obtain the post repolarization refractoriness(PRR) and coefficient a of the relationship between the pacing cycle length and FPD. [Results & Conclusions] Electropharmacological profiles of drugs were successfully characterized; namely, 1) the changes in the current threshold and conduction property provided important information of Na⁺ channel blocking kinetics, 2) the change of coefficient a reflected drug-induced inhibition of hERG K⁺ channel, 3) the PRR indicated the relative contribution of these drugs to Na⁺ and K⁺ channel blockade, and 4) L-type Ca²⁺ channel blocking action was obvious in the field potential waveform of the hiPSC-CMs sheets, which will help to predict whether the net balance of Ca²⁺ and K⁺ channel blockade of a drug is proarrhythmic or antiarrhythmic.

1-P-075

Geometrical and Constituent Heterogeneity Jeopardizes the Electrical Conduction on Atrial-like Cardiomyocytes Monolayer Derived from Human Induced Pluripotent Stem Cells.

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Background: The present study is to estimate the geometrical and constituent heterogeneity effects on electrical conduction on *in vitro* monolayer consisting of atrial-like cardiomyocytes (ALCMs) derived from human induced pluripotent stem cells (hiPSCs) and human atrial fibroblasts (HAFbs) under HF field stimuli (HFFS).

Method: We induced hiPSCs into ALCMs by adding all-trans retinoic acid (ATRA). The ALCMs and HAFbs were transferred in defined ratios on manually fabricated plate with geometrical transition. HFFS were delivered, and the electrical propagation was assessed by optical mapping.

Results: ATRA-treated CMs showed atrial specific properties compared to untreated CMs. HFFS preferentially induced impaired conduction on ALCMs with an abrupt geometrical transition, but not on ALCMs with uniform geometry. In addition, the co-culture of HAFbs with the ALCMs deteriorated the stability of electrical conduction than in mono-culture of ALCMs.

Conclusion: Geometrical heterogeneity under HFFS jeopardizes electrical conduction on *in vitro* ALCMs monolayer. Constituent heterogeneity represented by HAFbs contributes to the deterioration of the electrical conduction stability.

1-P-076

GPR120 deficient mice facilitate the hepatic fibrosis of nonalcoholic fatty liver disease

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GPR120/FFAR4 has been recognized as a functional fatty acid receptor and an attractive therapeutic target for metabolic diseases. Previously, we have demonstrated that GPR120/FFAR4 deficit (GPR120KO) mice facilitate an inflammatory response of the nonalcoholic fatty liver diseases (NAFLD) after short-term a 0.1% methionine and choline deficient high-fat (CDAHf) feeding compared to WT mice. In this study, we investigated whether GPR120KO mice after long-term CDAHf feedings induce the progression of nonalcoholic steatohepatitis (NASH). Mice fed with CDAHf diet for 6 weeks showed a significant increase in plasma aspartate transaminase and alanine transaminase levels, fatty deposition, inflammatory cell infiltration, and severe fibrosis. Both WT mice and GPR120KO mice fed CDAHf diet showed increment of the number of crown like structures and the immunoreactivity for F4/80 positive cells. However, GPR120KO mice significantly increased TGF- β mRNA collagen type 1 α mRNA in the liver compared to WT mice fed CDAHf diet, indicating that GPR120KO mice fed CDAHf diet showed more severe liver fibrosis than that of WT mice fed CDAHf diet. Therefore, our findings suggest that GPR120 signaling could be helpful as a regulatory factor of NAFLD/NASH progression.

1-P-077

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 $\alpha 7$ -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 $\alpha 7$ -nicotinic receptors in this model.

1-P-078

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-P-079

Ameliorative effect of a novel BET inhibitor CN210 on experimentally-induced murine Crohn's disease-like ileitis via inhibition of inflammatory cytokine expression

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The bromodomain and extra-terminal (BET) inhibitor have emerged as promising new cancer agents via regulation of epigenetic mechanism. Recent studies further demonstrate that BET inhibitors exhibit anti-inflammatory effects in animal models of various inflammatory diseases. In the present study, we examined the effect of BET inhibitor on inflammatory bowel disease (IBD) in experimentally-induced murine Crohn's disease (CD)-like ileitis models. Ileitis was induced in male C57BL/6 mice by subcutaneous administration of indomethacin and the ileum was examined 48 h later. CN210 was given orally 30 min before and 24 h after indomethacin administration. Further, the effect of CN210 on LPS-stimulated cytokine expression in cultured RAW264.7 cells. The administration of CN210 reduced the severity of indomethacin-induced ileitis in a dose-dependent manner. Indomethacin-induced upregulation of inflammatory cytokines such as TNF- α , IL-1 β and IL-6 was also significantly attenuated by administration of CN210. In RAW264.7 cells, LPS upregulated the expression of inflammatory cytokines, and this response was potently abrogated by CN210. These findings suggest that CN210 ameliorates indomethacin-induced ileitis via inhibition of inflammatory cytokine expression. Thus, CN210 is a novel candidate for the treatment with IBD including CD.

1-P-080

Leukotorien B4 receptor type 2 (BLT2) accelerates the healing of intestinal injury via promoting cell proliferation

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BLT2, a low-affinity leukotriene B4 (LTB4) receptor, is highly expressed in intestinal epithelial cells. Recently, 12(S)-hydroxyheptadeca-5Z,8E,10E-trienoic acid (12-HHT) was identified as an endogenous ligand for BLT2. However, the precise role of this receptor has not been fully understood. The present study investigated the role of BLT2 in the healing of intestinal injury using BLT2-deficient (BLT2KO) mice, transgenic mice with intestinal epithelium-specific overexpression of BLT2 (villin-BLT2-Tg), and murine epithelial cell line (YAMC). The intestinal injury was induced in mice by subcutaneous administration of indomethacin and the healing of injury was determined 48, 72 and 96 h later. The wound (diameter: 1 mm) was inflicted on monolayer of YAMC and wound closure was evaluated 6 h later. Further, cell proliferation was determined by WST-1 assay. The healing of indomethacin-induced intestinal injury was significantly delayed in BLT2KO mice when compared with wild-type (WT) mice. In contrast, villin-BLT2-Tg mice exhibited healing-promoting properties when compared with WT mice. In YAMC, CAY10583, a BLT2 agonist, concentration-dependently promoted wound repair and cell proliferation. The similar effect was observed by 12-HHT. These findings suggest that BLT2 accelerates the healing of intestinal injury. This effect is at least partly mediated via promotion of epithelial cell proliferation.

1-P-081

Construction of a mouse model for irritable bowel syndrome induced by early childhood social defeat stress

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Although irritable bowel syndrome (IBS) is the most common functional gastrointestinal disorder, the pathophysiology has poorly understood. Several studies demonstrated that the traumatic stress in early childhood increases the risk of IBS. The present study tried to construct a novel mouse model for IBS induced by early childhood social defeat stress. Four weeks old of male C57BL/6 mice were exposed to a trained aggressor mouse for 5-10 min daily for 10 days. After exposure to stress, these mice were maintained under normal conditions for 5 weeks. The state of early childhood social defeat stress was continued until 5 weeks after the exposure to the stress. The number of 5-HT- and CGRP-positive nerve fibers were significantly increased while the number of CD4-positive cells were significantly decreased in the colonic mucosa with early childhood social defeat stress. The visceromotor response to colorectal distention was significantly increased in stress mice compared with normal mice, indicating the development of colonic visceral hyperalgesia. The severity of visceral hypersensitivity was attenuated to the control level by TRPV1 antagonist BCTC. Taken together, these results suggest that early childhood social defeat stress induces IBS-associated visceral hyperalgesia in adulthood, probably via activation of TRPV1. Thus, this model may be useful for studies on the pathophysiology of stress-associated IBS.

1-P-082

Pattern analysis of osteoclastic bone resorption dynamics *in vivo*

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Osteoclasts are bone-resorbing giant polykaryons that differentiate from mononuclear macrophage/monocyte-lineage hematopoietic precursors. We have originally established an advanced imaging system for visualizing *in vivo* behavior of osteoclasts with an intravital two-photon excitation microscope. We also developed pH-activatable fluorescent probes to detect low pH areas on the bone surface and succeeded in visualizing areas where osteoclasts actually resorb bones *in vivo*. However, the spatiotemporal dynamics of acidification by osteoclasts remains unclear. In this study, we developed a novel image analysis system to evaluate the relationship between acidic regions and osteoclast dynamics. By means of this system, we found that the acidification by osteoclasts on the bone surface shows a characteristic pattern that is dependent on motility of osteoclasts. This approach would be beneficial for understanding the mechanism of osteoclastic bone resorption *in vivo* and would thus be useful for evaluating the efficacy of novel anti-bone-resorptive drugs.

1-P-083

Bone anabolic effects of MPMBP, a non-nitrogen-containing bisphosphonate, in young rats

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Disodium dihydrogen-4-[(methylthio) phenylthio] methanebisphosphonate (MPMBP) is a novel, non-nitrogen-containing BP with an antioxidant side chain. In this study, we compared the effects of MPMBP with those of zoledronate on bone remodeling in rats. MPMBP or zoledronate was subcutaneously injected every three days in neonatal or growing rats. The animals were euthanized after sequential labeling with tetracycline and calcein, and their tibias, femurs, jaw bones were harvested and examined. Bone morphometric analyses revealed that MPMBP increased the bone mass of the distal femur and proximal tibia. The zoledronate-treated neonatal rats showed reduced weight gain, suppression of longitudinal growth in the hind limbs, and a marked delay in tooth eruption, whereas the MPMBP-treated rats showed normal weight gain and eruption of teeth at the appropriate developmental stage. Further, the MPMBP-treated rats showed higher fluorescence intensity of calcein in their trabeculae, revealing that new mineralization occurred following treatment with MPMBP. In conclusion, our results show that MPMBP has a potent anabolic effect on bones, whereas zoledronate has severe adverse effects due to excessive inhibition of bone resorption and/or bone remodeling.

1-P-084

b-series gangliosides deficiency in mice resulted in the prevention of age-related bone loss

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Purpose: b-series gangliosides are involved in the regulation of cell growth, neurite extension, and apoptosis. However, little is known about their roles in bone metabolism. In this study, we investigated effects of deletion of b-series gangliosides in bone metabolism.

Material & Methods: We examined expression levels of b-series gangliosides (GD3, GD2, GD1b, and GT1b) in MC3T3E1 osteoblast-like cells, RAW264.7 pre-osteoclast, and primary bone marrow cells using flow cytometry. To determine whether b-series gangliosides are involved in bone metabolism, we analyzed bone phenotype of GD3 synthase-knockout (GD3S KO) mice lacking all b-series gangliosides using mCT.

Results & Conclusion: b-series gangliosides were not detected in MC3T3E1 cells. On the other hand, they were detectable in both RAW264.7 cells and primary bone marrow cells. However their expression was reduced after induction of osteoclastogenesis. No differences in bone phenotype between GD3S KO and wild type mice at the age of 15 weeks were detected. However, bone volume (BV/TV) in GD3S KO mice at the age of 40 weeks was higher than that in wild type mice. Correctively, these results suggest that b-series gangliosides may prevent age-related bone loss.

1-P-085

Novel measurement of femoral neckbone strength for OVX mice

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The number of patients suffering from osteoporosis increases year by year. Osteoporosis is characterized by decreased bone density and increases the risk of fracture. The femoral neck is the most possible part of the fracture. In this study, bone strength of ovariectomized osteopenia (OVX) model mouse was measured using a newly improved stage device for femoral neck fracture. **METHODS** Female ICR mice were divided into the sham and OVX groups. Eight weeks after surgery, right and left femurs were taken out. The right femur neck was set on a bone strength tester and the maximum breaking load (N) was measured. The left femur was measured for bone density using a CT. All results were regression analyzed with Stat View analysis software and their correlation was obtained. **RESULTS** In the right femur, N value of the OVX group showed a significant decrease compared to that of the sham group. The value of bone density of OVX group was also lower. Regression analysis of N value and bone density revealed a significant positive correlation coefficient in total bone density and cancellous bone density. **CONCLUSION** The present study indicates that the novel strength test of the femoral neck using the femoral neck fracture stage is useful for an evaluation of OVX model mice.

1-P-086

Interaction between interleukin-1 α and protease-activated receptor-2 expressions in human oral epithelial cells

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Protease-activated receptors (PARs) have a classical heptahelical structure within the plasma membrane and stimulate phosphoinositide turnover by coupling to G-protein. There are four known members of the receptor family of PARs: PAR-1, 3 and 4, which are activated by thrombin and gingipain, and PAR-2, which is activated by trypsin and mast cell tryptase. We previously demonstrated that IL-1 α increased ESE-3 mRNA expression through MEK1/2 pathway in human oral epithelial cells (HO-1-N-1 cells). The present study was undertaken to investigate the interaction between interleukin-1 α (IL-1 α) and PAR-2 expressions in HO-1-N-1 cells. The cells were cultured to semi-confluence and treated with IL-1 α or PAR-2 agonist for 6 - 24 hours. RNA was isolated from the cells, and IL-1 α and PARs expressions were analyzed by RT-PCR. To measure the amount of IL-1 α in cell culture supernatant, ELISA was performed according to the manufacturer's protocol using the Human IL-1 α Quantikine ELISA Kit (R&D systems, MN, USA). HO-1-N-1 cells showed PAR-1 and PAR-2 mRNA expression. PAR-2 agonist increased IL-1 α mRNA expression, and IL-1 α increased PAR-2 mRNA expression in the dose dependent manner at 6 hours in HO-1-N-1 cells. PAR-2 agonist increased extracellular IL-1 α level in HO-1-N-1 cells. These results suggest that IL-1 α and PAR-2 may play an important role in inflammatory oral mucosal disease.

1-P-087

Antibody drug for control of gingival epithelial cell adhesion

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Dental implant therapy is a highly effective treatment for recovering occlusion after tooth loss. An important factor in the success of dental implants is establishing strong osseointegration. If more epithelial cells migrate to the implant bone interface than mesenchymal stem cells, effective osseointegration may fail. Therefore, controlling epithelial cell adhesion and motility would be an effective strategy to increase the success rate of osseointegration. Laminin-332 is a major component of the basement membrane and is composed of three chains (α 3, β 3 and γ 2). It is well-known that laminin-332 regulates cellular functions such as adhesion, proliferation, apoptosis and differentiation. These biological functions depend on changes in the structural arrangement of laminin-332 by proteolytic cleavage. We focused on cleavage site of α 3 and developed antibodies that target the cleavage site. To investigate the influence of the monoclonal antibody on the cell adhesion function of epithelial cells for the α 3 chain of laminin-332, we compared it with the cell adhesion function of human epithelial cells from the Cas9-22 cell line. The monoclonal antibody significantly decreased cell adhesion for the laminin-332 α 3 chain when compared with no monoclonal antibody in both laminin-332 doses, 1 and 10 μ g/mL. We could propose that it would be possible that we change the biological function of laminin-332 to control cell adhesion for the purpose of regulating dental implant therapy.

Distribution of pathogenic oral bacteria in shimane

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Back ground: Pathogenic oral bacteria induces not only peridontitis and dental caries, but also contribute to causing systemic diseases. However, large-scale etiological study for characterizing regional distribution pattern of pathogenic bacteria is lacking.

Aim: To obtain data of distribution pattern of pathogenic bacteria in shimane prefecture.

Method: Saliva samples were collected from local residents of 7 area in shimane prefecture (1588 samples). Bacterial genome DNA was extracted. Then, following bacteria was detected by PCR; *P. gingivalis* (*P.g*), *T.denticola* (*T.d*), *T. forsythia* (*T.f*), *P. intermedia* (*P.i*), *A. actinomycetemcomitans* (*A.a*), *S. mutans* (*S.m*).

Results: Infection percentage of *T.f* and *A.a* was 90% and more in all area. In contrast, frequency of *T.d* infection was very low every area. There are regional differences in infection of *P.g*, *P.i*, *S.m*. Especially inland residents frequently had *P.g* compared to people in other area. One area showed outstanding low infection percentage in *S.m*. Number of people who had both *P.g* and *S.m* were almost same with number of *S.m*-infected people in each area.

Conclusion: Each area showed distinct infections pattern, suggesting that living environment affects the infection of oral pathogenic bacteria.

1-P-089

Evaluation of tumor infiltrating lymphocyte and cytokine/chemokine production with anti-PD-1 antibody treatment in mouse syngeneic models

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Cancer immunotherapy opened the new way of cancer therapy and is noticed as the fourth therapy. Consequently, we need a system that can evaluate effectiveness of immune checkpoint inhibitors.

In this study, we measured tumor infiltrating lymphocytes (TILs) and cytokine/chemokine productions which were triggered by anti-PD-1 antibody treatment in CT26WT (colon), LLC (lung) or B16F10 (melanoma)-bearing mice syngeneic models. Anti-PD-1 or control antibody were administered intraperitoneally twice a week for two weeks. The anti-tumor effect was evaluated by tumor volume. Furthermore, after isolation of TILs from tumor, the TILs population was analyzed by flowcytometer. The productions of cytokine/chemokine including IFN- γ , TNF- α and CCL5 were measured by Cytometric Bead Array (CBA) or AlphaLISA analysis.

As a result, changes in the response against antibody, lymphocyte distribution and cytokine/chemokine productions were confirmed in the anti-PD-1 antibody treatment group. The differences in immune function were observed in the three mouse tumor cell lines. The method for evaluating the effectiveness of immune checkpoint inhibitors was established by analyzing TILs and inflammatory mediators.

1-P-090

miR-200c-3p regulates invasive capacity in human oral squamous cell carcinoma microenvironment

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Recently, extracellular vesicles—particularly exosomes—have been recognized as intercellular communicators in the tumor microenvironment. As exosomic cargo, deregulated microRNAs (miRNAs) can shape the surrounding microenvironment in a cancer-dependent manner. Previous studies have shown inconsistent results regarding miR-200c-3p expression levels in OSCC cell lines, tissues, or serum—likely because of the heterogeneous characters of the specimen materials. For this reason, single-cell clone analyses are necessary to effectively assess the role of exosome-derived miRNAs on cells within the tumor microenvironment. In this study, we performed integrated microarray profiling to compare exosome-derived miRNA and exosome-treated cell-derived mRNA expression. Data were acquired from noninvasive SQUU-A and highly invasive SQUU-B tongue cancer cell clones derived from a single patient to determine candidate miRNAs that promote OSCC invasion. Matrigel invasion assays confirmed that hsa-miR-200c-3p was a key pro-invasion factor among six miRNA candidates. Consistently, silencing of the miR-200c-3p targets, CHD9 and WRN, significantly accelerated the invasive potential of SQUU-A cells. Thus, our data indicate that miR-200c-3p in exosomes derived from a highly invasive OSCC line can induce a similar phenotype in non-invasive counterparts.

1-P-091

Identification of tumor infiltrating lymphocyte subsets using the CT26WT tumor-bearing mouse model

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Immune checkpoint inhibitors such as anti-PD-1 antibody and anti-CTLA-4 antibody have recently been approved for the treatment of melanoma or non-small cell lung cancer. In this study, we examined an evaluation method for tumor infiltrating lymphocytes (TILs), which is one of the evaluation items of immune checkpoint inhibitors.

BALB/c mice were subcutaneously inoculated with CT26WT(mouse colorectal cancer cell line). They were allocated into anti-PD-1, anti-CTLA-4, or their combination treatment and the control groups. The drugs were administered intraperitoneally twice a week for two weeks. The tumor diameters were measured and tumor volumes were calculated. Observation and measurement were performed up to 14 days after the initiation of administration. Then, the tumor was excised and dispersed. TILs were isolated using CD45 microbeads, stained by fluorescently-conjugated antibodies and analyzed using flow cytometer.

As a result, the drugs changed the proportion of TILs subsets including regulatory T cells (Treg), CD8⁺ T cells, dendritic cells (DC) and myeloid-derived suppressor cells (MDSC). It is suggested that the evaluation system described above is useful for identification of TILs subsets in the CT26WT-bearing mouse model.

1-P-092

Improvement of tumor microenvironment induce macrophage activation in tumor bearing mouse.

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Tumor tissue environment is generally exposed to low oxygen, nutrition depletion and high interstitial pressure condition. These circumstances are caused by vascular hyper-permeability, irregular vascularization and immature vessels. We previously reported that prolyl hydroxylase inhibitor (PHDi) induced tumor blood vessel normalization and improved tumor microenvironment (TME) in tumor bearing mouse. In this study, we examined whether improvement of TME by PHDi elicit phenotypic alteration of tumor infiltrated immune cells, especially macrophage (Mf). Lewis lung carcinoma cells were transplanted subcutaneously. Mice were treated with PHDi intraperitoneally at day10 after tumor transplantation. Then tumor tissues were collected at day16 and analyzed immune cells by flowcytometry and immunofluorescence staining. we performed phagocytosis assay using sorted Mf from tumor tissue and bone derived Mf. Mf ratio in total leukocyte were significantly increased in PHDi treated tumor in both immunohistochemical and flowcytometric analysis. Lymphocyte ratio didn't change in PHDi treated tumor. Both in vivo and ex vivo experiments showed that phagocytosis ability of Mf increased about 1.5 folds in PHDi treated Mf. these Mfs may affect tumor progression.

1-P-093

Phospholipase C-related catalytically inactive protein modulates cytokinesis progression

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Phosphatidylinositol 4,5-bisphosphate [PI(4,5)P₂] is an important molecule for the progression of cytokinesis and accumulates the cleavage furrow during cytokinesis. Here, we investigated whether phospholipase C (PLC)-related catalytically inactive protein (PRIP), a metabolic modulator of PI(4,5)P₂, regulates PI(4,5)P₂-mediated cytokinesis. PRIP localized to the cleavage furrow during cytokinesis, and *PRIP*-knockdown HeLa cells displayed abnormal cytokinesis. Importantly, PI(4,5)P₂ accumulation at, and the localization of RhoA and phospho-myosin II regulatory light chain to, the cleavage furrow were reduced in the *PRIP*-knockdown cells. The overexpression of oculocerebrorenal syndrome of Lowe-1 (OCRL1), a phosphatidylinositol-5-phosphatase, in cells decreased PI(4,5)P₂ levels during early cytokinesis and showed cytokinesis abnormalities. However, these abnormal cytokinesis phenotypes were ameliorated by the co-expression of PRIP but not by a PI(4,5)P₂-unbound PRIP mutant. Collectively, PRIP is a component at the cleavage furrow to maintain PI(4,5)P₂ metabolism and regulates RhoA-dependent progression of cytokinesis.

1-P-094

O-GlcNAcylation stabilizes FOXM1 protein via suppression of its poly-ubiquitination

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O-GlcNAcylation is a post-translational modifications regulating dynamic intracellular signaling by two enzymes, OGT and OGA, which add and remove the modification, respectively. In many types of cancer cells, *O*-GlcNAcylation is elevated and contributes to the transformed phenotypes, but the molecular mechanisms is not fully understood. In this study, we examined *O*-GlcNAcylation-mediated cancer cell proliferation focusing on FOXM1 oncogenic transcription factor to regulate cell cycle. Elevated *O*-GlcNAcylation promoted cell proliferation in MKN45 gastric cancer cells, accompanying with increasing FOXM1 nuclear localization. FOXM1 was not *O*-GlcNAcyated, but was polyubiquitinated, which was reduced by elevated *O*-GlcNAcylation. We found some molecules involved in FOXM1 proteasomal degradation which are regulated by *O*-GlcNAcylation. One is GSK-3 β Ser/Thr kinase mediating FOXM1 phosphorylation to induce the ubiquitination (Ub). Elevated *O*-GlcNAcylation reduced GSK-3 β activity following increased FOXM1 protein. The other is FBXL2 ubiquitin E3 ligase mediating FOXM1 Ub. Elevated *O*-GlcNAcylation reduced FBXL2 protein via increased its Ub. These data suggest that *O*-GlcNAcylation-mediated FOXM1 stabilization could promote cancer progression.

1-P-095

Synergistic anti-cancer effects of PME-1 suppression and p53 activation on A549 cells

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Excessive phosphorylation of intracellular proteins is one of the causes of the development and malignant progression of cancer. Not a few molecular-targeted anticancer drugs have been developed to inhibit abnormal activation of limited number of kinases. Therefore, novel drugs development from this point of view is now facing difficulty. On the other hand, inovative drug discovery that targets "phosphatase activation", as a different angle of "kinase inhibition", has not been realized. Protein phosphatase 2A (PP2A) is an essential holoenzyme that is implicated as an important tumor suppressor based on its central role in phosphorylation-dependent signaling pathways. Protein phosphatase methyl-esterase (PME-1) catalyzes specifically the demethylation of PP2A catalytic subunit (PP2Ac). PME-1 also inhibits PP2A activity by directly binding to its phosphatase active site; the role as PP2A inhibitory protein. We revealed that PP2A inhibitory function, but not methyl-esterase activity, is important for tumor-promoting function of PME-1. We also found that PME-1 inhibition and p53 activation synergistically exert anti-cancer effects on human lung cancer cell line A549.

1-P-096

Drug repositioning using three-dimensional, MMP9 promoter activity-based anticancer drug screening

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Cancer is one of the most serious diseases all over the world, especially metastasis and drug resistance are leading causes of death. There is an urgent need to establish new strategies for drug discovery. Success in the drug discovery depends on the development of appropriate tumor models that correspond closely to native tumor situation. Matrix metalloproteinases (MMPs) represent the most prominent family of proteinases associated with tumorigenesis and are regulators of tumor milieu. The cancer stem cell model fits well with tumorigenesis, metastasis and drug resistance. We have shown that cancer cell aggregation led to hypoxic tumoroids with marked upregulation of reprogramming and stemness genes as increased cancer stem cell using a 3D culture system. In the present study, we established a novel MMP9 promoter-driven cell-based reporter system using a rapidly metastatic colon cancer cell in the 3D culture system that evaluates cancer stemness and invasiveness. We used a concept of drug repositioning-using known molecules for new indications. We selected several compounds with inhibition to both tumoroid formation and MMP9 promoter activity. One of the compounds inhibited primary tumor formation, invasion and metastasis.

1-P-097

Effects of inflammatory stimulation on the migratory ability of mouse Colon-26 cancer cells and B16-BL6 melanoma cells

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We previously reported that inflammatory stimulation reduced the tumor suppressor gene Programmed cell death 4 (Pdc4) protein expression level and enhanced the metastatic ability of human colon cancer cells. In this study, we investigated the effect of 12-*O*-tetradecanoylphorbol 13-acetate (TPA) stimulation on the expression levels of Pdc4 protein and migratory ability in mouse Colon-26 cancer cells and B16-BL6 melanoma cells.

The expression level of Pdc4 protein in Colon-26 cells treated with TPA significantly decreased in a concentration-dependent manner. On the other hand, Pdc4 protein in B16-BL6 cells treated with TPA significantly increased in a concentration-dependent manner. In addition, the migratory ability of Colon-26 cells treated with TPA were significantly increased, but the migration of B16-BL6 cells treated with TPA were significantly decreased. Furthermore, we measured the melanin content of the cells and tyrosinase activity as indices of activation of Protein Kinase C (PKC). The melanin content and tyrosinase activity were significantly decreased in B16-BL6 cells treated with TPA.

These results indicated that Pdc4 might be a negative regulator in the migratory ability of Colon-26 and B16-BL6 cells. Although the activation of PKC promoted degradation of Pdc4 in Colon-26 cells, it did not function in B16-BL6 cells.

1-P-098

Reactive oxygen species-independent cytotoxic effects of menthol in lung cancer cell line, A549

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Menthol has a cooling effect via TRPM8 activation resulting in a relaxation, an anti-inflammation and an analgesic by the inhalation and the topical application. Previously, we demonstrated that menthol induced cytotoxicity in different ways dependent on its concentrations in lung cancer cell line, A549. However, the detail mechanisms of the cytotoxic action are unclear. The expression of molecules related to cytotoxicity was detected by using western blotting. Flow cytometrical analyses were performed to detect apoptosis, intracellular reactive oxygen species (ROS) and mitochondrial membrane potential. The expressions of some cyclins and cyclin-dependent kinases were suppressed by menthol (1 mM). At 2 mM, menthol evoked apoptosis Ca^{2+} -independently concomitant with an increase of intracellular levels of ROS. N-acetyl cysteine failed to inhibit the menthol-induced apoptosis. Menthol rapidly decreased the mitochondrial membrane potential. These results suggest that menthol induces ROS-independent cytotoxicity despite the ROS production. Dysfunction of mitochondria may be involved in apoptotic action of menthol in A549.

1-P-099

Ceramide nanoliposomes as a MLKL-dependent, necroptosis-inducing, chemotherapeutic reagent in cancer

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Ceramides are bioactive lipids that mediate cell death in cancer cells and ceramide-based therapy is now being tested in dose-escalating phase 1 clinical trials as a cancer treatment. The most representative ceramide formulation is ceramide nanoliposomes (CNL) that have been preclinically studied. However, the effect of CNL in ovarian cancer still remains an open question. We now investigate the therapeutic efficacy and signaling mechanisms of CNL in ovarian cancer. Treatment of ovarian cancer cells with CNL decreased cell viability in a dose-dependent manner. Importantly, CNL-treated cancer cells died with programmed necrosis (necroptosis), but not apoptosis. Mechanistically, dying SKOV3 ovarian cancer cells exhibit activation of pseudokinase mixed lineage kinase domain-like (MLKL) as evidenced by oligomerization in necroptosis. In addition, inhibition of MLKL, but not upstream RIP kinases, abolished CNL-induced cell death. In a cell-free system, ceramide was revealed to interact recombinant MLKL. Those results suggest CNL exhibited a cytotoxic effect by inducing MLKL-dependent necroptosis. In clinical studies, relapse-free survival was significantly extended in high MLKL mRNA expression group of patients with breast cancer, demonstrating correlation of MLKL expression with good prognosis. Taken together, our studies give insight into pharmacotherapeutic significance of necroptosis-inducing reagents in cancer treatment.

1-P-100

Establishment of new monoclonal antibodies against HEG1, a new mesothelioma marker

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Malignant mesothelioma (MM) is a fatal tumor caused by past exposure to asbestos. The absence of highly specific markers for MM has served an obstacle for its diagnosis and therapy. We previously produced a monoclonal antibody (mAb) against MM, SKM9-2. We also discovered that SKM9-2 specifically binds to protein HEG homolog 1 (HEG1) that is a novel mucin-like membrane protein. This specificity of SKM9-2 to MM was due to the recognition of HEG1 glycopeptide containing a sialylated glycan. However, there was no mAb that bound to HEG1 glycosylation-independently. We produced new mAbs against HEG1 in this study. Purified partial HEG1 expressed in mammalian cells was immunized in mice or rats. After antigen-immunized spleen cells were isolated from mice or rats, the cells were fused with myeloma cells by electrical cell fusion. Hybridomas were screened by ELISA, western blotting or flow cytometry. Through the screening of about 10,000 clones, we obtained more than 10 clones of anti-HEG1 mAb. These antibodies could bind to HEG1 glycosylation-independently. We also obtained two mAbs that can be used in immunohistochemistry. We will investigate the non-glycosylated HEG1 expression in normal tissues.

1-P-101

Evaluation of drug efficacy *in vivo* and *in vitro* using patient-derived xenograft (PDX)

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To improve the success rate of anti-cancer drug development, clinically relevant tumor models are needed. Patient-derived xenograft (PDX) tumor, which is generated by direct implantation of human tumor into immunodeficient mice, has been well-accepted as a more clinically relevant model than cell line-derived xenograft (CDX) tumor.

We have been developing both *in vivo* and *in vitro* screening systems for anti-cancer drugs using PDX tumors that had been established in the National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN). In this study, we confirmed anti-cancer drug efficacy using PDX *in vivo* and *in vitro*.

In the *in vivo* study, the PDX tumor was subcutaneously transplanted into immunodeficient mice, and a control group and drug group were set. Gemcitabine or 5-FU was administered once or twice a week, and observation was carried out until 28 days after starting administration. Anti-cancer effect was evaluated by tumor volume.

In the *in vitro* study, three-dimensional culture was performed using dispersed cells derived from PDX tumor. Drugs were added to the medium and the cell viability was confirmed by ATP assay.

In both studies, the effect of anti-cancer drug was confirmed, and an evaluation system *in vivo* and *in vitro* using PDX could be established.

1-P-102

Effects of various 5-HT3 receptor antagonists on cisplatin-induced acute kidney injury

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OBJECTIVE: □Cisplatin (CDDP) is known to frequently cause nausea and vomiting, renal injury as a side effect. There are many kinds of 5-HT3 receptor antagonists, which are one of the basic antiemetic drugs for nausea and vomiting by cancer chemotherapy, such as the first generation ondansetron, granisetron, the second generation palonosetron. It is suggested that ondansetron may be a risk factor for the onset of CDDP-induced acute kidney injury (AKI). Therefore, in this study, the effect of various 5-HT3 receptor antagonists on CDDP-induced renal injury was examined.

METHODS: C57BL/6 mice were intraperitoneally administered with CDDP. Renal function was evaluated by serum creatinine and blood urea nitrogen. Histological damage in the cortex of HE-stained kidney sections was scored. Various 5-HT3 receptor antagonists were administered 30 minutes before administration of CDDP.

RESULTS: CDDP-induced renal injury got significantly worse by pre-administration of ondansetron, but not by pretreatment of palonosetron compared with cisplatin alone group.

CONCLUSIONS: These results suggest that the second generation 5-HT3 receptor antagonist may have less effect on CDDP-induced AKI than the first generation.

1-P-103

An attempt to clarify the mechanisms underlying the modulatory effects of Docosahexaenoic acid on human glial glutamate transporter

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Glial L-glutamate (L-Glu) transporter EAAT2 removes L-Glu at the synaptic cleft, thereby maintaining an efficient neurotransmission. Docosahexaenoic acid (DHA) is a major constituent of astrocyte membrane phospholipids and is released after L-Glu stimulation, however, the effects of DHA on L-Glu transporter have not been fully clarified. We studied the effects of DHA on EAAT2 electrophysiologically. Bath-applied DHA increased the amplitude of EAAT2 currents, but had no effects on EAAT1, another glial EAAT subtype. DHA has a negative charged carboxyl group that is deprotonated in a pH dependent manner. When the extracellular pH was decreased, the enhancement of EAAT2 by DHA was disappeared. No chargeable DHA analogue had no effects on EAAT2 currents, suggesting the negative charge is important. We identified which part of EAAT2 is important for the effects of DHA using EAAT1/2 chimeras. By substituting the transport domain of EAAT1 by that of EAAT2, the effects of DHA on EAAT1 were turned out to be enhancement, suggesting this region is important for the enhancement of EAAT2 current by DHA. Currently, we are identifying the essential region for the effect of DHA.

1-P-104

Inhibition of cell proliferation by L-theanine transported into cells via an L-glutamine transporter Slc38a1

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L-Theanine is an amino acid ingredient in green tea with a structural analogy to glutamine and is suggested to be taken up into the cells via an L-glutamine transporter Slc38a1. Recently, the oral intake of L-theanine is expected to suppress anxiety, sleep disturbance, and cognitive impairment. Some reports showed that L-theanine possesses anticancer activities against some cancers. Here, it was investigated whether L-theanine inhibits cell proliferation and its mechanism is via Slc38a1. L-Theanine inhibited cell proliferation in mouse motor neuron cell line (NSC-34), mouse neuroblastoma cell line (Neuro 2A) and human neuroblastoma cell line (SH-SY5Y) in a dose- and time-dependent manner. However, it had little effect in human brain glioblastoma cell line (U-251 MG), mouse astrocyte cell line (C8-D1A), mouse brain endothelial cell line (bEnd3) and human umbilical vein endothelial cells (HUVEC). There was a positive correlation between the L-theanine-dependent inhibition of cell proliferation and the expression level of Slc38a1 mRNA ($r^2 = \sim 0.6$). Therefore, it was suggested that in these cell lines, the suppressive effect on cell proliferation was caused by L-theanine which was taken up into the cells via Slc38a1.

1-P-105

Characterization of pre-incubation inhibitory effects of JPH203 on L-type amino acid transporter 1 function.

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BACKGROUND/AIM: JPH203 is a novel anti-cancer drug targeting L-type amino acid transporter 1 (LAT1), which plays a key role in essential amino acid uptake in cancer cells. While the co-incubation inhibitory effect of JPH203 has been characterized in conventional uptake assay, its pre-incubation inhibitory effects remain undetermined. Therefore, we aimed to characterize pre-incubation inhibitory effects of JPH203 on LAT1 function. **METHODS:** Pre-incubation effects were examined by leucine uptake assay using LAT1-positive human colon cancer HT-29 cells. **RESULTS:** In time-dependency analysis, pre-incubation of HT-29 cells with 10 μ M JPH203 for 30, 60, and 120 min resulted in a significant decrease of the leucine uptake activity (42%, 32%, and 28% of those obtained from the control cells, respectively). Similarly, in concentration-dependency analysis, pre-incubation of the cells with JPH203 (1, 10, and 30 μ M for 120 min) decreased the activity level to 68%, 25%, and 3% of those of the control cells, respectively. **CONCLUSION:** We have identified potent pre-incubation inhibitory effects of JPH203 on LAT1 function. Combination effects of pre- and co-incubation inhibitory effects are currently under examination.

1-P-106

Comparative phosphoproteomics between non-competitive and competitive inhibitions of L-type amino acid transporter 1 in cancer cells.

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L-type amino acid transporter 1 (LAT1) is a major essential amino acid transporter in cancer. Leucine is one of the LAT1 substrates and acts as a signaling molecule to regulate cell growth and proliferation by stimulating mTORC1 pathway. Recently, several groups, include us, reported that inhibition of LAT1 suppresses the growth of cancer cells. The inhibition of LAT1 is a promising procedure for cancer therapeutics. Many researchers have been developing LAT1 inhibitors, but all of them inhibits LAT1 in a competitive manner. Due to abundant amounts of amino acids in living organisms, the competitive inhibition seems not to be the most practical way to control the function of the amino acid transporter *in vivo*. Thus, we have developed a series of non-competitive inhibitors of LAT1, called OKY compounds.

In this study, we found that a classical LAT1 competitive inhibitor, BCH, and one of the OKY compounds suppressed mTORC1 pathway in the same manner, and both compounds even made similar effects on comprehensive phosphoproteomes *in vitro*. These results indicate that the inhibition of LAT1 causes the same effect on cellular signaling in both competitive and non-competitive manners.

1-P-107

A specific PET tracer for L-type amino acid transporter 1 (LAT1) differentiates the function of transporter in tumors from that in inflammatory lesions

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LAT1 (SLC7A9) is a large-neutral amino acid transporter that forms heterodimer with CD98hc to function at the plasma membrane. LAT1 is a target for cancer diagnosis and therapy due to its important roles on cancer cell growth. PET imaging using FDG is a common cancer diagnosis. Yet, high accumulation of FDG in inflammatory lesions causes false positive results. In contrast, we and others reported that LAT1 selective PET tracers including ¹⁸F-FAMT (3-fluoro-L-a-methyl-tyrosine) were highly accumulated in tumors but not in inflammatory lesions.

Here, we demonstrated that FAMT was taken up by tumors but not by inflammatory lesions in animal models indicating no LAT1 function in inflammatory lesions, despite similar LAT1 expression in tumors and inflammatory lesions. LAT1 formed complex with CD98hc at the plasma membrane in tumors. However, LAT1 retained as monomer inside the cells of inflammatory lesions while CD98hc existed as dimer. Membrane proteome showed other SLC7 transporters in the inflammatory lesions whereas LAT1 dominated in tumor. Taken all together, in inflammatory lesions unlike tumors, LAT1 exists as a monomer inside of the cells and has no function at the plasma membrane, and likelihood CD98hc forms complex with SLC7 family beside LAT1. Our results suggest the different roles of LAT1 in tumors and inflammatory lesions.

1-P-108

Identification of interacting proteins for a liver-specific butyrate transporter OAT7

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Short chain fatty acids, including acetate, propionate and butyrate, have been shown to regulate various metabolic processes such as energy and lipid metabolism. Of these short chain fatty acids, butyrate has been identified as a ligand for GPR41/43, both of which are known to stimulate GLP-1 production from L cells in the intestine. Thus, the butyrate blood levels would correlate with GLP-1 production and one of the determinants of the butyrate level is its uptake/release by the liver. Human liver-specific organic anion transporter 7 (OAT7), expressed at the hepatic sinusoidal membrane, has been functionally characterized as an exchange transporter of butyrate with sulfate conjugated steroids.

The purpose of this study is to clarify the regulation of butyrate transport function. As a first step we attempted to identify OAT7 interacting proteins by yeast two-hybrid and immunoprecipitation followed by mass spectrometry in human liver cell lines, HepG2 and Huh7. One of the proteins identified by yeast two-hybrid, PDZK1/NHERF3, was likely to regulate OAT7 function. We are currently seeking other regulatory proteins for OAT7 by proteomic analysis of the immunoprecipitant.

1-P-109

Exploring the mechanism of uric acid lowering effect of iguratimod

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Iguratimod (IGU) is a disease-modifying antirheumatic drug, whose activity is mainly ascribed to the inhibition of NF- κ B pathway. Interestingly, we have noticed that the serum uric acid (UA) levels were decreased in 22% of rheumatoid arthritis patients taking IGU. Since there have been no reports of IGU affecting UA, we decided to explore how IGU lowers the serum UA level.

We first examined whether IGU inhibits the xanthine oxidase (XO) activity. Xanthine and XO were incubated with the drug and the production of UA was measured by absorbance at 290 nm. UA production was not inhibited by IGU. Next, we examined whether IGU or its metabolites inhibit the UA transporters. As M3 and M4 are the major metabolites in the urine, we examined the inhibitory activities of IGU and these metabolites against the major UA transporters, GLUT9 and URAT1, in the xenopus oocytes expression system. IGU and M4 inhibited the URAT1, and their IC_{50} s were $12.61 \pm 0.97 \mu M$ and $120.07 \pm 26.69 \mu M$, respectively. However, the estimated maximal concentrations of these compounds in the proximal tubule were 4 - 7 times lower than the IC_{50} .

In conclusion, UA lowering effect of IGU is not likely to be explained by its or its metabolite's direct interaction with XO, URAT1, or GLUT9.

1-P-110

GRP78 promotes ERK activation through endothelin type B receptor

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Endothelin (ET)-1 is involved in various diseases, including cancer, hypertension, atherosclerosis, diabetes, and fibrotic diseases, although ET-1 is originally identified as endothelium-derived vasoconstrictile peptide. ET receptors belong to the class A of G protein-coupled receptor, and consist of ET type A receptor (ET_AR) and ET type B receptor (ET_BR). ET_AR and ET_BR generally exhibit the opposite responses, although many exceptions exist. Here, we attempted to identify ET_AR or ET_BR specific binding proteins to understand difference of ET_AR- and ET_BR-mediated responses upon ET-1 stimulation. We found that GRP78 exhibited a stronger binding affinity toward ET_BR than ET_AR. Overexpression of GRP78 promotes ET_BR-mediated ERK activation. In addition, the silencing of *GRP78* suppressed ET_BR-mediated ERK activation. On the other hand, ET_BR can localize GRP78 to cell periphery. Our results suggest that interaction of ET_BR with GRP78 affects the ERK activation and GRP78 localization.

1-P-111

Molecular characterization of urate transport via paracellular route.

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Since charged molecule cannot permeate cell membrane, urate movement across the epithelial cell layer has to be transcellularly by carrier or endocytosis/exocytosis, or paracellularly. We have reported that the paracellular route is the major urate transport pathway across the blood-placental barrier. In this study, the mechanism of urate paracellular transport was investigated in several epithelial cell lines. Very little urate passed through MDCK and LLC-PK1 cell layers. Interestingly, one Caco-2 cell line was urate non-permeable while another Caco-2 cell line was found to be urate-permeable. Urate paracellular movement across the Caco-2 cell layer was partially inhibited by DIDS, which inhibits chloride transport.

Detection and quantification of claudin proteins that are important for paracellular transport of ions were performed by LC/MS. Claudin 1, 3, 4, 6, 7 and 12 were detected in urate-permeable cell lines, BeWo and Caco-2 cells. However, overexpression of these claudins in MDCK cells did not increase urate paracellular transport.

In conclusion, overexpression of single claudin was not sufficient to make urate-non-permeable cell become urate permeable.

1-P-112

Time-dependent inhibition demonstrated across multiple classes of uptake transporters

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Purpose: To identify novel time-dependent (TD) inhibitors of uptake transporters *in vitro*.

Methods: HEK293 cells overexpressing uptake transporters OATP1B1/1B3, OAT1/3, OCT1/2, and MATE1/2-K were used to determine IC₅₀ values of corresponding inhibitors with or without 3 hours of preincubation. A total of 64 transporter-inhibitor combinations were analyzed. A shift in IC₅₀ greater than 2.5-fold (i.e., IC₅₀ with preincubation $\leq 0.4 \times$ IC₅₀ without preincubation) was considered relevant. In addition, transwell permeability of the inhibitors across a low efflux MDCK cell (MDCK-LE) monolayer was measured.

Results: TD inhibition was observed, albeit with different frequencies, across all classes of uptake transporters investigated. The proportion of inhibitors tested positive with at least one member of a cognate transporter pair was 3/5 for OATP1B1/1B3, 1/10 for OAT1/3, 6/9 for OCT1/2, and 1/8 for MATE1/2-K. In particular, ledipasvir, an antiviral previously not recognized as an OCT inhibitor, was shown to potently suppress both OCT1 and 2 upon preincubation (IC₅₀ with preincubation: 0.15 μ M and 74.3 μ M, respectively). MDCK-LE permeability of the inhibitors ranged between 0.012 and 16.9×10^{-6} cm/s, and compounds with low to medium P_{app} ($\leq 5 \times 10^{-6}$ cm/s) were more likely to show TD behavior, as such compounds were involved in 10/15, or 66.7%, of observed cases of TD inhibition. However, the association between MDCK-LE permeability and TD effect was not statistically significant. Among inhibitors that were non-substrates of their respective transporters, the magnitude of IC₅₀ shift correlated positively with cLogP (Spearman's $r = 0.43$, $P = 0.008$) and molecular weight ($r = 0.67$, $P < 0.0001$).

Conclusion: Since TD behavior was seen not only in OATPs but also in OATs, OCTs, and MATEs, the phenomenon of TD transporter inhibition seems to extend beyond OATPs. Non-substrate

1-P-113

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-P-114

Effect of Nyoshinsan on decreased voluntary activity of OVX mice

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Nyoshinsan is one of the kampo medicine used in the treatment for menopausal disorder. We have already reported that ovariectomized (OVX) rats were seen in the decreases of voluntary activity at the dark time and serotonin level in amygdala (Behav. Brain. Res. 227(1)1-6(2012)). In this study, we examined effects of Nyosihnsan on a voluntary activity in OVX mice. The female ICR mice of 9-week old were OVX or sham operation. Either Nyoshinsan (750 mg/kg/day, p.o.) or water were administered to each group for 8 weeks (6 times/week) starting from 8 weeks after OVX. The voluntary activity of mice was evaluated by using an activity sensor (model NS-AS01 neuroscience, Inc.) at dark time (19:00-7:00). Moreover, RNA expression level of tryptophan hydroxylase (TPH) was measured in hippocampus and prefrontal cortex by Real-time PCR. In the result, Nyoshinsan significantly prevented decreased voluntary activity at dark periods induced by OVX. In addition Nyoshinsan suppressed down-regulation of TPH mRNA expression level induced by OVX. These results revealed that these OVX induced despair-like behaviors were improved by administration of Nyoshinsan. Moreover, it was also thought that the voluntary activity mediated by serotonin level in hippocampus.

1-P-115

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-P-116

Assessment of the responses to convulsants in hiPSC-derived neurons and mouse acute brain slices

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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. In the reliability of the evaluation system, it is important to compare the response of hiPSC-neuron with the response in the living brain. In this study, we compared the responses to typical convulsants in cultured hiPSC-derived cortical neuronal network compared with mouse acute hippocampal slice. 4-Aminopyridine (4-AP), Pilocarpine, Picrotoxin (PTX), Pentylentetrazol (PTZ), and negative control acetaminophen, DMSO were tested in both samples using MEA system. The change of number of burst, inter burst interval (IBI) and CV of IBI in negative control and DMSO administration were almost same both samples. Although the number of burst increased both sample in convulsants administration, dose-dependency were difference between hiPSC-derived neurons and acute slices. However, CV of IBI were decreased dose-dependently both samples. In three convulsants, it was found that the hiPS neuron and the acute slice exhibit partially similar firing patterns.

1-P-117

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-P-118

Mechanism of organophosphate paraoxon-induced kinetic tremor in mice

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Organophosphates (OPs) inhibit cholinesterase and hyperactivate the acetylcholinergic nervous system in the brain, causing motor excitement (e.g., tremor and seizures). However, the mechanism underlying the motor excitement by OPs remains unknown. Here, we performed behavioral and immunohistochemical studies in mice to investigate the tremorigenic mechanism of paraoxon, an active metabolite of parathion. Treating animals with paraoxon (0.15-0.6 mg/kg, i.p.) elicited kinetic tremor in a dose-dependent manner. Expressional analysis of Fos protein, a biomarker of neural excitation, revealed that a tremorigenic dose of paraoxon (0.6 mg/kg) significantly and region-specifically elevated Fos expression in the dorsolateral striatum (dlST) and the inferior olive (IO) among 48 brain regions examined. Moderate to slight increases in Fos expression was also observed in the cerebral cortex, hippocampus, nucleus accumbens, medial striatum, globus pallidus, medial habenula, and solitary nucleus, while these changes were not statistically significant. Paraoxon-induced tremor was inhibited by the nicotinic acetylcholine (nACh) receptor antagonist mecamylamine (MEC), but not affected by the muscarinic acetylcholine receptor antagonist trihexyphenidyl (THP). In addition, paraoxon-induced Fos expression in the dlST and IO was also antagonized by MEC, but not by THP. The present results suggest that OPs elicit kinetic tremor primarily by activating dlST and IO neurons via nACh receptors.

1-P-119

Study of healing effects of rebamipide on an acetic acid-induced oral stomatitis in Syrian golden hamsters

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[Background] We studied whether rebamipide would exert healing effects in an acetic acid-induced model of oral stomatitis in animals either untreated or pretreated with 5-FU. [Methods] The oral stomatitis model was prepared by anesthetizing male Golden Syrian hamsters. The center of the cheek pouch was then exteriorized and sandwiched between ring forceps 5 mm in inside diameter, followed by submucosal injection of 25 μ L of 10% acetic acid solution through the ring forceps. [Results] In animals un-pretreated with 5-FU, rebamipide at 3 mg/mL was shown to significantly inhibit acetic acid-induced oral stomatitis in comparison with animals that did not receive rebamipide. The area of oral injury in 5-FU-pretreated animals was significantly greater than that in animals not pretreated with 5-FU. The recovery time in 5-FU-pretreated group was longer than that in the 5-FU-un-pretreated group. In 5-FU-pretreated animals, the area of oral injury was significantly reduced in those that received 3 mg/mL rebamipide in comparison with those that did not receive rebamipide. [Conclusion] The present findings suggest that rebamipide has healing effects on acetic acid-induced oral stomatitis in animals both pretreated and un-pretreated with 5-FU.

1-P-120

Bisphosphonates induce osteoblastic cell death through the inhibition of autophagic flux

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Extracting teeth of patients treated with bisphosphonates (BPs) occasionally induces the necrosis of jaw (BRONJ), but the cause of disease is still unclear. I found out that the intracellular BP of osteoblastic cells was gradually accumulated in lysosomes. In the present study, I investigated the mechanism of osteoblastic cell death induced by BPs. MC3T3-E1 cells were used as osteoblastic cells. The uptake of BP into cells was observed by fluorescent pamidronate. Apoptosis was evaluated by PSVue 480 and CellEvent caspase 3/7. Formation of autophagosome and autolysosome was observed using DAPI and DAPI respectively. Autophagosome and autolysosome were frequently observed in the cytosol of normal MC3T3-E1 cells. On the other hands, autolysosomes of cells treated with BPs were gradually accumulated around nuclei. The mitochondria of BPs-treated cells were decreased in both response and quantity. Moreover, BPs shrank nuclei of cells. After a while phosphatidylserine (early apoptotic marker) exposed on cell membranes was detected, and finally the activation of caspase 3 was observed. Mitochondria in cells treated with BPs were not co-localized with autolysosomes. These results suggest that BPs may accumulate autolysosome around nuclei of osteoblastic cells, and induce apoptosis due to inhibiting autophagic flux.

1-P-121

Elucidation of molecular mechanism for detoxification of cigarette smoke gas phase by antioxidants

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Unsaturated carbonyl compounds such as acrolein (ACR) and methyl vinyl ketone (MVK) are major cytotoxic factors in the gas phase extract of cigarette smoke. ACR and MVK induce cell membrane damage and cell death through protein kinase C and NADPH oxidases. We have previously reported that several antioxidants such as reduced glutathione (GSH) and N-acetylcysteine (NAC) can suppress ACR- and MVK-induced cell membrane damage and cell death, although the molecular mechanism has remained to be clarified. In this study, we have elucidated the mechanism for suppression of ACR- and MVK-induced cell injury by antioxidants. The molecules with thiol group such as GSH, NAC, and cysteine effectively suppressed cell membrane damage and cell death induced by cigarette smoke gas phase, ACR, and MVK. The results of HPLC and MS showed that GSH and NAC directly reacted with ACR and MVK. Cysteine and cysteine derivatives suppressed ACR-induced protein carbonylation. Current results suggest that GSH, NAC, and cysteine directly reacted with ACR and MVK, and suppressed unsaturated carbonyl compounds-induced cell damage by preventing protein carbonylation.

1-P-122

An early carcinogenic marker for chemically induced hepatic carcinogenesis in rats

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Toxicogenomics project is a Japanese government and pharmaceutical companies joint project to gather the gene expression data after exposure to about 150 compounds to rats in vivo for up to 28 days, rats and humans in vitro. The data are available for public use on the internet as "Open TG-GATES". Analysing the microarray data from "Open TG-GATES", genes fall in the following criteria were selected 1) increase more than 2 times, 2) the expression changes occur within 7 days from the initial dose, 3) those changes occur with several carcinogenic agents, 4) not or minimally expressed in the normal liver, and 5) related to proliferation or apoptosis. Several genes fell in the criteria and we focused on one of them, p75-NTR associated cell death executor (NADE) gene. NADE is known to bind to some partner proteins in the cell and in most cases, cells die by apoptosis. We further investigated whether NADE is a promising early carcinogenic marker using carcinogen exposed rats. Sprague-Dawley rats were treated with diethylnitrosamine for up to 8 weeks followed by up to another 11 weeks of no treatment. The liver was excised to obtain RNA and protein samples and paraffin embedded tissue sections. The NADE expression pattern correlates with the development and growth of cancerous cells. It is likely that the NADE plays a role in chemically induced carcinogenesis in the rat liver and it can be a good early gene marker of carcinogen.

1-P-123

Establishment of dimethylnitrosamine (DMN) induced rat liver fibrosis model and effect of human mesenchymal stem cells from the bone marrow (hMSC) on the fibrosis model

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Purpose: This study was aimed to develop a DMN-induced liver fibrosis model in rats and to evaluate effects of hMSC on the model.

Methods: DMN was given i.p. to SD rats 3 times weekly for 4 weeks. hMSC was injected at the day after the last DMN treatment. Blood was collected once a week to perform blood biochemistry test. Liver was removed from week 3 to 6 to observe the content of fibrosis and to perform histopathological examination.

Results: DMN treated rats showed a progressive increase in plasma ASAT, ALAT, γ GT, Tbil and hyaluronan from week 1 to 4, a significant decrease in plasma Alb and a significant increase in plasma ALP, a significant increase in fibrosis area ratio and hydroxyproline of liver compared to the non-treatment rats. Histopathological examination indicated DMN induced inflammation and liver fibrosis from 3rd week after start of DMN treatment. Administration of hMSC did not affect the model rats under the present conditions.

Conclusion: A rat liver fibrosis model was developed under the present study conditions and the model can be reproduced consistently within a relatively short period of time and can be used to assess the drug efficacy of potential anti-fibrotic agents.

1-P-124

Early postnatal elimination of slow-type motor neurons induces progressive muscle atrophy and kinetic tremor

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Fast muscles (white muscles) are innervated by fast-type motor neurons (MNs); slow muscles (red muscles) are by slow-type MNs. VACHT-Cre is a Cre-driver mouse line that can direct DNA recombination in postnatal slow-type motor neurons (Misawa et al., *genesis*, 54, 568-572, 2016). To selectively eliminate slow-type motor neurons, VACHT-Cre mice were crossbred with NSE-DTA mice in which diphtheria toxin A (DTA) was expressed after the loxP-site excision. The VACHT-Cre; NSE-DTA mice (delta SlowMN mice) were born normally but showed progressive body weight loss, tremor and reduced life-span (average life, ca. 40 weeks). Muscular atrophy was evident in red muscles including the soleus and diaphragm, however white muscles were normal. Complex fiber-type transitions were observed in each muscles. The delta SlowMN mice showed hunched back posture (kyphosis) possibly by reduced trunk muscles. The tremor was kinetic in nature and most conspicuous in head and neck. The observed abnormalities were largely different from those observed in mutant SOD1-expressed ALS model mice in which fast-type motor neurons are known to be preferentially affected. The delta SlowMN mice could be a novel MN disease model characterized in proximal muscle atrophy, tremor and bulbar involvement.

1-P-125

Study of the effects of nintedanib ethanesulfonate on bleomycin-induced murine pulmonary fibrosis model

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Idiopathic pulmonary fibrosis (IPF) is a refractory disease that progresses from alveolar damage and inflammation to interstitial fibrosis. In this study, we confirmed the progress of the pathological condition of pulmonary fibrosis model in mice and investigated the effects of IPF therapeutic agent nintedanib ethanesulfonate (ofev).

Bleomycin was intravenously administered to 9-10 weeks old male ICR mice for 5 days. To confirm the progress of the pathological condition, the mice were euthanized after 28, 42 and 56 days of initial administration of bleomycin, lungs were collected and weighed. The content of lung hydroxyproline (HP) and fibrosis area were measured, and histopathological examination were performed. Ofev was administered to mice by gavage at 3 and 10 mg/kg once a day for 42 consecutive days.

Increases in relative lung weight and lung HP content were noted in mice given bleomycin for 5 consecutive days intravenously. The formation of fibrotic lesions was confirmed and increases in the fibrosis regions were observed over time, histopathologically.

In addition, orally administration of ofev at 10 mg/kg/day to mice for 42 days, suppression of increase in lung relative weight and lung HP content was confirmed.

1-P-126

Decrease in pancreatic islets size in mice lacking p13, a mitochondria-localized protein

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p13 is mitochondrial protein originally identified as one of the proteins down-regulated in pancreatic islets of diabetic mice. Previously, we generated transgenic mice overexpressing p13 in pancreatic beta cells and demonstrated that overexpressed p13 exerts multiple beneficial effects against type 2 diabetes, such as the increase in islets size and insulin secretion. Here, we performed further histological analysis using pancreatic tissue slices of p13-knockout mice in order to investigate the roles of endogenous p13 in pancreatic morphogenesis and function. Although there was no significant difference in islet number, the average islet size was decreased by 42% in p13-knockout mice compared with wild-type mice. Notably, very small islets ($<0.001 \text{ mm}^2$) were observed specifically in p13-knockout mice. The present results suggest that p13 plays a critical role in islets morphogenesis.

1-P-127

Analysis of immune cells using redox-state monitoring mice

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Reactive oxygen species (ROS) is produced in immune cells during immune responses and is necessary for host defense and inflammation. Furthermore, ROS acts as signals for gene expression and required for T cell proliferation and activation. While low levels of ROS play important roles in cell activation, high levels of ROS induce significant damage to cells. To monitor redox state in living cells we generated transgenic mice expressing a green fluorescent protein (roGFP) whose fluorescence varies with redox state (J Invest Dermatol. 34, 1701-1709, 2014). CRO mice, measuring the redox state of whole cells and MRO mice, measuring the redox state of mitochondria were generated. Immune cells were isolated from CRO and MRO mice, and treated with hydrogen peroxide (oxidized state) or DTT (reduced state) to evaluate the maximum oxidation and reduction value in immune cells. Next, splenocytes isolated from CRO and MRO mice were stained with cell surface markers, and the redox state in various types of immune cells was analyzed by flow cytometry. We found that T cells were more oxidized than B cells in both CRO and MRO. This system should be a powerful and convenient tool for analyzing redox state in various types of immune cells.

1-P-128

***In vivo* genome-editing of serotonin-1A receptor gene using Cas9 knock-in mice**

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Although accumulating evidence suggests that serotonin (5-HT) controls emotional behaviors and cognition via acting on its receptors, it is not clear that the receptor subtype responsible for the specific function. Fourteen pharmacologically distinct 5-HT receptor subtypes have been identified, but some of them lack any selective ligands because of their similarity. It is expensive and impractical to obtain knock-out mice for all the subtypes. Therefore we attempted to knock out 5-HT receptor genes by injecting virus vectors expressing sgRNA to adult Cas9 knock-in mice. This approach would provide easier and low-cost knock out of 5-HT receptor genes in specific cell types and brain regions. First, we targeted 5-HT_{1A} receptors in the dorsal raphe nucleus (DRN) because the phenotype of 5-HT_{1A} knock-out mice has been clarified. A 5-HT_{1A} agonist, 8-OH-DPAT induced hypothermia is attenuated by the injection of viral vector expressing sgRNA for 5-HT_{1A} gene into DRN of Cas9 knock-in mice. DNA sequencing and mismatch cleavage assay detected the target gene editing. Immunohistochemical analysis revealed that most of the virally induced Cas9 were expressed in GAD-positive GABAergic neurons.

1-P-129

Quantification of oxygen tension in bone marrow using intravital two-photon phosphorescence lifetime imaging

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Osteoclasts are derived from the monocyte-macrophage lineage of the bone marrow and are bone-resorbing cells essential for bone homeostasis. Osteoclasts contain more mitochondria and require more oxygen to support the energy demands associated with bone resorption. However, local oxygen distribution within bone marrow has not been completely understood. Intravital two-photon microscopy is a powerful tool for investigating biological processes in live animals. In this study, we performed two-photon phosphorescence lifetime imaging to characterize the oxygen tension in osteoclasts.

Two-photon-enhanced phosphorescent probe, iridium complex (BTPDM1), was injected into knock-in mice in which EGFP is expressed in osteoclast lineage cells. The emissive triplet state of the BTPDM1 is sensitive to local oxygen tension. By measuring the phosphorescence from EGFP positive cells, oxygen tension in osteoclasts was determined. Furthermore, we quantitatively analyzed changes in oxygen tension in osteoclasts by measuring the phosphorescence decay time in EGFP positive cells. Thus, we have succeeded in detecting oxygen tension *in vivo*. This method is applicable to various cell types for determining local oxygen tension inside bone marrow.

1-P-130

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-P-131

Rapid measurement of plasma concentration of a molecular targeted agent, imatinib, with diamond sensor.

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Imatinib is a classical molecular targeted anticancer agent that specifically suppresses a hyperactivated tyrosine kinase and used for treatment of the patients with chronic myeloid leukemia. The application has been recently expanded to gastrointestinal stromal tumor. This drug is initially administered to all the patient at the same dose without normalization by parameters of the individuals such as the body surface area and weight and the variation in metabolism. Therefore, it is difficult to expect the effects of the therapy. Optimization of the administration protocol for individual patients requires immediate determination of plasma level of the compounds at clinical site. To address this issue, we show a simple procedure with an electrochemical approach. The sensor consists of boron-doped diamond electrode, a state-of-the-art material that elicits more stable reaction than classical materials. We examined guinea-pig plasma containing imatinib at different concentrations. With the procedure, each measurement was completed in 35 sec. This method was sensitive to the drug of 0.3 to 10 μ M, the range included in a therapeutic window. The methodology described here may contribute to advances in pharmacotherapies for cancer.

1-P-132

KANPHOS (Kinase-Associated Phospho-Signaling) Platform - A comprehensive database for kinase-associated neural phosphorylation signaling

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Protein phosphorylation is a major and essential post-translational modification in eukaryotic cells that plays a critical role in various cellular processes. While recent advances in mass spectrometry based proteomics allowed us to identify approximately 200,000 phosphorylation sites, it is not fully understood which sites are phosphorylated by a specific kinase and which extracellular stimuli regulate the protein phosphorylation via intracellular signaling cascades. Recently, we have developed an in vitro approach termed the kinase-interacting substrate screening (KISS) method and an in vivo approach termed kinase-oriented substrate screening (KIOSS) method. Using KIOSS method, we analyzed the phosphorylation signals downstream of dopamine in mouse striatal slices, and found that about 100 proteins including ion channels and transcription factors were phosphorylated probably by PKA or MAPK. Here, we present an on-line database system which provides the phosphorylation signals identified by our KISS and KIOSS methods as well as those previously reported in the literature. The database system and its web portal, named KANPHOS (Kinase-Associated PHOspho-Signaling), were built based on the Next Generation XooNIps. We also demonstrate how to retrieve proteins and pathways in striatal medium-sized spiny neurons modulated by extracellular dopaminergic stimulation.

1-P-133

Development of turn-on fluorescent tag system for live-cell imaging

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Fluorescence labeling of proteins is a key technique in live-cell imaging. We have developed a turn-on fluorescence labeling technique named DeQODE (De-Quenching of organic dye emission) tag technology. In this technology, a specially designed quenched fluorescent probe called QODE (Quenched organic dye emission) probe is used. The QODE probe consists of a fluorescent moiety and a quenching moiety. The DeQODE tag is the single chain antibody (scFv) that binds the quenching moiety to turn on fluorescence emission of the QODE probe. We here report a new QODE probe employing arylazopyrazoles moiety (AAP) as a quencher, and silicon rhodamine as a fluorophore. To produce the DeQODE tag for the new QODE probe, we obtained scFv clones against AAP. Two clones were found to express in a mammalian cell line 293T. The cells expressing these clones showed strong fluorescence in the presence of the QODE probe. Washing out of the free QODE probe was unnecessary for high-contrast imaging. This property is advantageous for application for in vivo imaging and high content drug screening.

1-P-134

Design and constructs of the “All-in-One” type single double-conditional shRNA expression vectors for a spatio-temporal gene and/or cell targeting

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An important advance in the RNA interference (RNAi) field was the discovery that plasmid vector-mediated expression of short hairpin RNA (shRNA) can substitute for synthetic small interference RNA (siRNA) *in vitro* and *in vivo*. But the constitutive and/or ubiquitous knockdown of target gene by this method has still limited the utility, especially if the inhibition of target gene leads to lethality, which prevents *in vivo* functional analysis. To overcome these limitations, the time (or period)- and cell (or tissue)- specific regulation of shRNA expression should have been required as the double-conditional mice. Conventional protocols for such an artificial regulation of gene expression *in vivo* are based on the tetracycline-controlled reversible system for the time- and the Cre-loxP system for cell-specificities respectively. The double-conditional transgenic mice are expected by mating their two lines of genetic engineered mice at last. Therefore, we have designed the "All-in-One" type single double-conditional shRNA expression vectors having both systems within a single vector format to make the transgenic mice with double-conditional RNAi without much effort.

1-P-135

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.