1-P-001

Development of a fluorescent labeling method for the live-cell superresolution imaging of synaptic molecules.

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Synaptic transmission is regulated by nanoscale assembly of synaptic molecules. Stimulated emission depletion (STED) microscopy is a modality of superresolution microscopy that allows visualizing such nanoscale molecular distribution. However, photobleaching severely limits time-series imaging by STED microscopy in living neurons. In this study, we aimed to develop a fluorescence labeling method that circumvents the limitation due to photo-bleaching in STED microscopy. Our method is based on a fusion protein tag that reversibly binds a small organic dye and turns on its fluorescence emission. We reasoned that this reversibility in binding enables everlasting imaging because the bleached dye should be continuously displaced by a fresh dye. Accordingly, we prepared expression constructs of the protein tag fused with synaptic molecules, RimBP2, CAST, and Rim1a. When expressed in cultured hippocampal neurons, the synaptic localization of these molecules was visualized under a standard fluorescence microscope. We successfully performed STED imaging of RimBP2 and CAST with the spatial resolution of less than 100 nm at 0.2 Hz for 10 min. Thus, our fluorescence labeling method enables live STED microscopy in neurons which will be useful to unveil the dynamic feature of nanoscale molecular distribution underlying synaptic function.

1-P-002

Effects of orexin A on excitatory synaptic transmission in the rat nucleus tractus solitarius

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Orexin A (OX_A) and orexin B (OX_B) are neuropeptides produced in the lateral hypothalamus and show widespread distribution in the CNS. These neuropeptides play essential roles in sleep-wake control and metabolism regulation. Recently cardiovascular function is also reported. Orexin-1 receptor (OX_1R) and orexin-2 receptor (OX_2R) were identified and the former is reported to be selective for OX_A and the latter is nonselective for OX_A and OX_B. The nucleus tractus solitarius (NTS) expresses OX_1R and OX_2R. OX_B shows excitatory effects on excitatory synaptic transmission but the effects of OX_A is not examined. This report examined the effects of OX_A on excitatory synaptic transmission in the rat NTS neurons using a slice patch-clamp technique.

OX_A (1 micro M) increased the frequency of spontaneous EPSCs (sEPSCs) in with or without TCS-OX2-29 (10 micro M:OX_2R blocker). Prior application of L-NAME (100 micro M) blocked the effect of OX_A under the presence of TCS-OX2-29. Contrary, OX_A (1 micro M) decreased the amplitude of tractus solitarius (TS) evoked EPSCs (eEPSCs) in with or without TCS-OX2-29 (10 micro M). Prior application of L-NAME (100 micro M) did not block the effect of OX_A under the presence of TCS-OX2-29. The action of OX_A on both sEPSC and eEPSC seemed to be presynaptic.

These results suggest that the activation of OX_1R in the NTS facilitates spontaneous excitatory synaptic transmission through NO production and inhibits TS-evoked excitatory synaptic transmission through distinct mechanism other than NO production.

1-P-003

Distinct sensitivities to bafilomycin and brefeldin to high potassium-evoked DOPA and dopamine release from PC12 cells

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L-3,4-Dihydroxyphenylalanine (DOPA) is a precursor of a neurotransmitter dopamine (DA), and synthetized by tyrosine hydroxylase in the cytoplasm of catecholaminergic neurons. We proposed that DOPA is a neurotransmitter. Neurotransmitter storage and release are regulated by vesicular transport system. However, it is unknown whether there are vesicular transport system(s) for DOPA. In this study, we examined whether depolarizing stimuli release DOPA from cultured PC12 cells. Then we performed characterization of the evoked release of DOPA and DA. High K^+(45 mM) induced the release of DOPA and DA. Both the releases were decreased by deprivation of extracellular Ca^{2+}. We next examined the effects of bafilomycin, a vacuolar type H^+-ATPase inhibitor, on the release, and found that bafilomycin inhibited the release of DA, but not DOPA release. In contrast, brefeldin A, which is known to induce Golgi complex di-assemble and its redistribution into the endoplasmic reticulum, thereby exerting its inhibitory action on secretion, suppressed the DOPA release and to a lesser extent DA release. These findings suggest that the release of DOPA may occur through a secretion pathway distinct from that for DA in PC12 cells.

1-P-004

Elucidation of the mechanism of feeding control by VMH-PACAP via DMH-galanin in the hypothalamus

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We have previously shown that PACAP in the ventromedial hypothalamic nucleus (VMH) enhances feeding at night and after fasting, but inhibits feeding during daytime. On the other hand, the neuropeptide galanin is also shown to be highly expressed in the hypothalamus and involved in feeding regulation. In this study, we investigated the possible involvement of VMH-PACAP in the dorsomedial hypothalamic nucleus (DMH)-galanin signaling for mouse feeding behavior. The expression of galanin in the hypothalamus was significantly increased by fasting, but this increase was cancelled in PACAP knockout mice. Furthermore, overexpression of PACAP in the VMH increased the expression of galanin, while knockdown of PACAP in the VMH decreased the expression of galanin, indicating that the expression of galanin in the hypothalamus might be regulated by PACAP in the VMH. Therefore, we expressed synaptophysin-EGFP chimeric protein in PACAP neurons in the VMH, and visualized the neural projections to the hypothalamic region where galanin was highly expressed. Strong EGFP signal was observed in the DMH, suggesting that PACAP-expressing neurons in the VMH projected to the DMH. Furthermore, in the DMH, immunostaining of galanin showed that galanin expression increased with fasting, but this was not observed in PACAP knockout mice. When galanin in the DMH was knocked down by shRNA treatment, food intake at night and after fasting was decreased, whereas food intake during daytime was increased, as shown in the PACAP knockout mice. These results suggested that VMH-PACAP may regulate mouse feeding behavior through DMH-galanin.

1-P-005

Role of fatty acid-binding protein 3 on the development of the medial prefrontal cortex

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Polyunsaturated fatty acids (PUFAs) are essential for brain development and function. Increasing evidence has shown that an imbalance of PUFAs is associated with various human psychiatric disorders, including autism and schizophrenia. Fatty acid-binding proteins (FABPs), cellular chaperones of PUFAs, are involved in their intracellular trafficking, signal transduction, and gene transcription. Previously, we showed that FABP3 is strongly expressed in the parvalbumin (PV)-positive inhibitory interneurons (PV interneurons) of the adult mouse anterior cingulate cortex (ACC), which is a part of rodent medial prefrontal cortex (mPFC) and is important for the coordination of cognitive and emotional behaviors. Although the expression of FABP3 becomes evident after birth, the function of FABP3 is largely unknown in postnatal brain. In particular, the effects of FABP3 deletion in the ACC PV interneurons are unclear. In this study, we first confirmed that FABP3 was expressed in the PV interneurons of postnatal day 14 (P14) ACC. PV synapse density increased in the P14 ACC of *Fabp3* KO mice, whereas the number of PV interneurons remained unchanged. These results suggest that FABP3 is involved in the formation of inhibitory synapse in the ACC.

1-P-006

Super-resolved 3D-STED microscopy reveals a layer-specific increase in excitatory synapses in the hippocampal CA1 region of a model mouse of autism spectrum disorder, *Neuroligin-3* KO mice

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The chemical synapse transmits information from a neuron to another neuron in the neuronal network in the brain. The efficacy of synaptic transmission changes by modifying the number or size of synapses dynamically in cognitive functions. Thus, morphological analyses of synapses are of particular importance in neuroscience research. In the current study, we applied super-resolved three-dimensional stimulated emission depletion (3D-STED) microscopy for the morphological analyses of synapses. This approach allowed us to estimate the precise number of excitatory and inhibitory synapses in the mouse hippocampal tissue. Using this method, we discovered a region-specific increase in excitatory synapses in a model mouse of autism spectrum disorder, *Neuroligin-3* KO. We detected an increase in excitatory synapses at the stratum oriens of hippocampal area CA1, although such an increase was not detected by conventional confocal microscopy. Our approach to estimating the synapse number will open a new field in developmental neuroscience.

1-P-007

Diverse intracellular signaling pathways mediate the effects of neurotensin on the excitability of type II neurons in the rat dorsolateral bed nucleus of the stria terminalis

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The effects of neurotensin (NTS) on the excitability of type II neurons in the rat dorsolateral bed nucleus of the stria terminalis (dlBNST) were examined using whole-cell patch-clamp electrophysiology. Bath-application of NTS depolarized type II dlBNST neurons. Analyses of the steady-state *I–V* relationships implied that the depolarizing effect of NTS is due to potassium conductance blocking. The depolarizing effect of NTS was abolished in the presence of a PLC inhibitor, but not affected by a protein kinase C inhibitor. In the presence of a CaMKII inhibitor, NTS showed depolarizing effects via the increase in non-selective cation conductance in addition to the decrease in potassium conductance. Unexpectedly, in the presence of a PKA inhibitor, NTS hyperpolarized type II dlBNST neurons. These results reveal that diverse signaling pathways mediate the effects of NTS on the excitability of type II dlBNST neurons. The possible intracellular signaling mechanism is that activation of the Adenylate cyclase-cAMP-PKA pathway exerts depolarizing effects on type II dlBNST neurons by decreasing potassium conductance and increasing non-selective cation conductance, whereas activation of the CaMKII pathway exerts hyperpolarizing effects on dlBNST neurons by decreasing non-selective cation conductance.

1-P-008

The effect of a miR-96-5p inhibitor delivery to brain using microbubbles and ultrasound technology on neuroprotection and microglial activation

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Glutathione (GSH) is an important antioxidant that plays a critical role in neuroprotection. GSH depletion in neuron induces oxidative stress promoting neuronal damage, which is regarded as a hallmark of the early stage in some neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease. The neuronal GSH levels are mainly regulated by excitatory amino acid carrier 1 (EAAC1) and its inhibitory protein, glutamate transporter-associated protein 3-18 (GTRAP3-18). In this study, we found that GTRAP3-18 levels were increased by the up-regulation of the microRNA miR-96-5p, which has been reported to decrease EAAC1 levels in our previous study. We also discovered that neuro-oncological ventral antigen 1 (NOVA1) is an intermediate protein for GTRAP3-18 expression via miR-96-5p. Moreover, we show that the administration of a miR-96-5p-inhibiting nucleic acid to living mice by a drug delivery system using microbubbles and ultrasound decreased the levels of GTRAP3-18 via NOVA1, while increased the levels of both EAAC1 and GSH in the mouse brain. Although the treatment of microbubbles and ultrasound itself increased microglial activation, the administration of miR-96-5p inhibitor decreased its activation. These findings suggest that the delivery of a miR-96-5p inhibitor to the brain would efficiently increase the neuroprotective activity by increasing GSH levels via EAAC1, GTRAP3-18 and NOVA1.

1-P-009

Promotion of cysteine-dependent glutathione synthesis by paraxanthine

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Caffeine (1,3,7-trimethylxanthine) consumption reduces the risk of neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. Several lines of evidence have suggested the neuroprotective effect of caffeine, however, the precise mechanism is still unclear in the central nervous system. Here, we suggest a mechanism that a caffeine derivative, paraxanthine (1,7-dimethylxanthine), promotes cysteine uptake and thereby enhances the synthesis of an antioxidant, glutathione (GSH), in neurons. We previously showed that paraxanthine, a major metabolite of caffeine, promotes cysteine uptake in mouse hippocampus slices. In this study, we examined the effect of paraxanthine on GSH synthesis in HEK293 cells. HEK293 cells were treated with 0, 10 and 100 μM of paraxanthine in addition to cysteine for 30 min and the GSH levels were subsequently detected by CMFDA, a fluorescent GSH marker, using fluorescent microscopy. Paraxanthine increased GSH levels in HEK293 cells at the concentration of 100 μM. These results suggest that paraxanthine promotes cysteine uptake leading to GSH synthesis in HEK293 cells.

1-P-010

Anatomical identification of segregated network modules of the retrosplenial cortex along the cingulate cortex

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The cingulate cortex, a brain region located from the genu of the corpus callosum to the splenium of the corpus callosum, is anatomically divided into anterior and posterior cingulate cortices (ACC and PCC, respectively). The ACC is involved in processing information for motor-related information and decision making, whereas the retrosplenial cortex (RSC), a region of the PCC, is involved in processing information such as memory and navigation. Accumulating evidence suggests that the ACC and RSC form neural circuits and integrate information, such as memory, navigation, and sensorimotor information, as well as contribute to symptoms of Alzheimer's disease. However, what information the RSC integrates from its presynaptic network and conveys to the ACC remains unknown. The purpose of this study is to identify a presynaptic network of ACC-projecting RSC neurons. Here we labeled ACC-projecting neurons in the anterior and posterior RSC (ACC-RSCa neurons and ACC-RSCp neurons, respectively) and their presynaptic neurons using adeno-associated viral vectors and rabies viral vectors. ACC-RSCa neurons and ACC-RSCp neurons received particular inputs among RSCa inputs and RSCp inputs, respectively. These results suggest that RSCa and RSCp neurons organize functionally segregated modules to convey particular information to the ACC.

1-P-011

Spatiotemporal dynamics of extracellular ADO revealed by genetically encoded ADO sensor *in situ* brain slice experiments.

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Adenosine (ADO) controls neuronal excitability in memory and sleep. It is believed that ADO level is regulated by degradation of extracellular ATP directly released from neurons and glia. However, it is still controversial how extracellular ADO is regulated. One of the problems is lack of the methods to evaluate its spatiotemporal dynamics. To understand how ADO is produced in the brain, we established a novel method to visualize extracellular ADO. We first used an adeno-associated virus to express GRABAdo, a genetically encoded ADO sensor, in hippocampal astrocytes. In acute hippocampal slices, we applied the Schaffer collateral electrical stimulation (30-900 pulses, 40 Hz, 0.1 mA) to induce ADO in response to neuronal activities. The ADO level was globally increased in a stimulus-dependent manner and was gradually reduced to baseline following termination of the electrical stimulation. Since microglia expresses enzymes to metabolize ATP, we investigated the role of microglia in the ADO elevation by microglia depletion in mice fed a diet containing PLX 5622. Surprisingly, microglia depletion completely abolished the increases of the ADO. The data suggest that microglia may be a major regulator of extracellular ATP metabolism to produce ADO. The method can be useful to study neuron-glia interactions via ADO pathway.

1-P-012

Fetal hypoxia caused decrease of gliogenesis-related gene expression in rat

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Fetal hypoxia (e.g., ischemia, threatened abortion) is one of the risk factors for neurodevelopmental disorder. We previously announced that exposure of prenatal hypoxic stress showed neurodevelopmental disease-like phenotype in rat and glial fibrillary acidic protein-positive cells were decrease in the rat cingulate cortex and hippocampus. In this study, we performed the qPCR and TUNEL staining to investigate the influence of fetal hypoxic stress in the brain. In gene expression analysis, fetal brain after hypoxic stress showed decrease of gliogenesis-related gene, especially Notch signal downstream genes were remarkedly decrease. In addition, one of these gene expression remained decrease in neonatal rat brain receiving prenatal hypoxic stress. Furthermore, TUNEL-positive cell expression was unchanged by prenatal hypoxia.

These results indicated that prenatal hypoxia decreased the expression of Notch signal downstream gene in both prenatal and neonatal brain without affecting cell death.

1-P-013

Region-specific astrocyte-microglia interaction promotes rotenone-induced dopaminergic neurodegeneration

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Pesticide exposure, such as rotenone or paraquat, increases the risk of Parkinson's disease. It is established that the inhibition of mitochondrial complex I is involved in rotenone-induced dopaminergic neurotoxicity. However, the mechanism underlying selective dopaminergic neurotoxicity by rotenone exposure remains unknown. Recently, we demonstrated astrocyte-microglia interaction promoted rotenone-induced non-cell-autonomous dopaminergic neurodegeneration. In this study, we examined involvement of region-specific glial crosstalk in rotenone neurotoxicity. We prepared mesencephalic neuronal culture and glial cell culture (astrocyte+microglia) from mesencephalon or striatum of SD rats embryos at 15 days of gestation. Direct treatment of mesencephalic neuronal cultures with rotenone failed to decrease dopaminergic neurons. Dopaminergic neurotoxicity was induced by treatment with conditioned media from rotenone-treated mesencephalic, but not striatal, glial cells. Furthermore, level of an antioxidant metallothionein-1 was reduced in the conditioned media from mesencephalic, but not striatal, glial cells following rotenone treatment. These results suggest that region-specific astrocyte-microglia interaction could play an important role in rotenone-induced dopaminergic neurodegeneration.

1-P-014

PDGF-BB-stimulated brain pericytes release the mediators related to blood-brain barrier dysfunction.

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Blood-brain barrier (BBB) dysfunction is observed in brain injury including traumatic brain injury (TBI) and stroke. Brain pericytes as well as brain endothelial cells are BBB-constituting cell types and release mediators related to BBB dysfunction under the inflammatory conditions. In the brain injury, the expression levels of PDGFRβ and PDGF-BB, a ligand for PDGFRβ, were increased in brain pericytes at the BBB and brain parenchyma in TBI model mouse, respectively. However, it has not been well known whether activated PDGFRβ signaling in brain pericytes results in the development and deterioration of the brain injury-evoked BBB dysfunction. We, therefore, evaluated whether PDGF-BB-stimulated brain pericytes release mediators associated with BBB dysfunction including matrix metalloproteinase (MMP)-9 and IL-6. Brain pericytes obtained from rat brains were incubated with PDGF-BB (2-20 ng/mL) for 24 h. PDGF-BB treatment significantly increased MMP-9 and IL-6 release from brain pericytes. Furthermore, the downregulation of PDGFRβ signaling with imatinib attenuated PDGF-BB-stimulated MMP-9 and IL-6 release. These findings suggested that brain pericytes may be a key player in the occurrence of brain injury-induced BBB dysfunctions.

1-P-015

Postnatal establishment of CNS border-associated macrophages involves Irf8 and Mafb

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The central nervous system (CNS) carries a variety of immune cells including macrophages, which can be divided based on their localization: microglia are found in the parenchyma, whereas the CNS interfaces such as meninges and perivascular spaces host CNS border-associated macrophages (CAMs). To date, in contrast to that of microglia, the nature of CAMs is poorly understood. In the present study, we investigated the distribution of CAMs during development and revealed previously-unappreciated kinetics of CAM subpopulation. Furthermore, we identified Irf8 and Mafb as crucial transcription factors for the establishment of CAMs, as well as microglia. Together, our data reveal a novel process and molecular machinery for the establishment of CNS macrophage subsets during development.

1-P-016

Effects of fluorocitrate, a selective inhibitor of astrocyte metabolism, on pentylenetetrazole-induced seizures

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Astrocytes regulate neural excitability via the spatial potassium (K^+) buffering mediated by inwardly rectifying Kir4.1 channels. In this study, we examined the effects of fluorocitrate (FC), a selective inhibitor of the astrocyte TCA cycle, on the expression of astrocytic Kir4.1 channels and the seizure susceptibility to pentylenetetrazole (PTZ) in rats. Microinjection of FC (1 nmol) into the right lateral ventricle caused no spontaneous seizures, but the number of the glial fibrillary acidic protein (GFAP)-positive astrocytes was decreased in the perirhinal-ectorhinal cortex, piriform cortex (PirC), basolateral amygdala (BLP), CA2 area and dentate gyrus (DG) of hippocampus. In addition, FC reduced Kir4.1 expression in PirC, CA2, and DG. When the susceptibility of rats to PTZ (40 mg/kg, i.p.)-induced seizures was evaluated, a significant increase in seizure intensity, an increased seizure incidence, and a tendency of delay in seizure onset were observed following the pretreatment of rats with FC (1 nmol, i.c.v.). Furthermore, PTZ-induced expression of Fos protein, a biological marker of neural excitation, was significantly increased in BLP, basomedial amygdala, and DG by the pretreatment with FC. These results suggest that the dysfunction of astrocytic Kir4.1 channels by FC elevates the susceptibility to PTZ seizures via hyperactivating the amygdala and hippocampal neurons.

1-P-017

HIF1α as a potential regulator of Schwann cell differentiation in peripheral nerve.

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Myelin sheath is essential for the rapid propagation of action potentials and proper functions of the nervous system. In the peripheral nervous system, it has been well-known that Schwann cells generate myelin sheath through several stepwise differentiation processes, but the detailed mechanism to regulate Schwann cell differentiation remains unclear. Previously, we demonstrated the involvement of hypoxia inducible factor 1 α (HIF1 α) in the regulation of peripheral myelination. To further investigate the role of HIF1 α in peripheral myelination, we used a conditional knock-out (cKO) mice lacking HIF1 α gene in myelinating Schwann cells. An in vitro myelination assay revealed that hypoxic treatment facilitated myelination in wild-type control but not cKO. Unexpectedly, peripheral myelin of cKO mice was formed normally without significant motor deficits. Next, we examined the remyelination in post-injury nerves. In cKO mice, the frequency of uncompacted myelin in regenerating nerve was higher than control mice. cKO mice also exhibited a delayed recovery in a sensory-motor function assessment. The comprehensive analysis of HIF1 α regulated gene expression in Schwann cells using a Chromatin integration labeling followed by sequencing technique revealed that HIF1 α stabilization by a transient hypoxic treatment increased the expression of several genes associated with Schwann cell differentiation. These findings suggested that HIF1 α might be involved in peripheral remyelination after nerve injury through regulation of Schwann cell differentiation.

1-P-018

ミクログリアにおけるミトコンドリア機能不全はミトコンドリア DNA 漏出と TBK1-IRF3 シグナルの活性増強を介して炎症が誘発する interferon-β の産生を増強する

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Background: Inflammation in central nerve system (CNS) is involved in onset and exacerbation of neurodegenerative diseases. Microglia play an important role in the CNS as main immune cells. We have reported that mitochondrial dysfunction by rotenone, an electron transporter chain I inhibitor, enhanced lipopolysaccharide (LPS)-induced interferon-β (IFN-β) production in microglia. However, the molecular mechanisms remain unclear. Therefore, the current study investigated the influence of mitochondrial dysfunction in intracellular signalings.
Method: BV2 cells, a mouse microglial cell line, were treated by rotenone for 24 hours to impair the mitochondrial function. The expression level of IFN-β was analyzed by real-time PCR and ELISA. The phosphorylated protein level was evaluated by Western blotting. To deplete mitochondrial DNA (mtDNA), BV2 cells were treated with ethidium bromide for 4 days.
Results: Mitochondrial dysfunction enhanced activation of interferon regulatory factor 3 (IRF3) by LPS. In addition, either depletion of mtDNA or treatment with TANK-binding kinase 1 (TBK1) inhibitor significantly blocked the LPS-induced IRF3 phosphorylation and IFN-β production in rotenone-treated BV2 cells.
Conclusions: We found that mitochondrial dysfunction enhanced IFN-β production through mtDNA and TBK1-IRF3 signaling.

1-P-019

Expression of equilibrative nucleoside transporters and activity regulation by hydrogen peroxide in cultured astrocytes

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We have found that cultured differentiated astrocytes pretreated with *N*^{6}, 2'-*O*-dibutyryladenosine 3',5'-cyclic monophosphate (DBcAMP), a permeable analogue of cAMP, incorporate thymidine, but not uridine, via nucleoside transporters including equilibrative nucleoside transporters (ENTs) into TCA insoluble fraction for repair on DNA injury in the presence of hydrogen peroxide (H_2O_2) at an early time, and these phenomena are specific in differentiated astrocytes, but not undifferentiated astrocytes and neurons.

We studied expression of ENTs in cultured astrocytes by RT-PCR, western blot analysis and immunocytochemistry. We could confirm ENT1, that is hypersensitive nucleoside transporter, and ENT2, that is low-sensitive nucleoside transporter in cultured astrocytes by RT-PCR and western blot analysis. This time, astrocytes ware double-stained by anti-GFAP antibody and anti-ENT3 antibody. We could confirm ENT3, that is assumed to be presented in lysosome, in cultured astrocytes co-stained by GFAP.

H_2O_2-induced thymidine incorporation into cultured astrocytes decreased by S-(4-Nitrobenzyl)-6-thioinosine (NBMPR), dilazep and dipyridamole, ENT inhibitors, at micromolar concentrations but not nanomolar concentrations, so thymidine was incorporated via ENT2, but not ENT1.

These results indicate that ENTs expressed in membrane and lysosome could relate with H_2O_2-induced thymidine incorporation and DNA repair in cultured astrocytes.

1-P-020

Involvement of microglial TRPV4 on glial scar formation

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Glial scar is a dense scar tissue that forms at the site of injury in the central nervous system. Although it is known that microglia, which are immune cells in the brain, are important in the formation of glial scar, the molecular mechanism is still unclear. It has been reported that the expression of TRPV4, a multistimulus receptor, is commonly upregulated in various injured areas. In this study, we focused on TRPV4 in microglia to understand the mechanism of glial scar formation. First, to investigate the effect of microglial TRPV4 on glial scar formation, we used a glial scar model using cultured hippocampal sections. Microglia-specific knockdown of TRPV4 reduced GFAP density in astrocytes, the main component of glial scar. Thus, microglial TRPV4 promotes glial scar formation. The abundance of TRPV4 in microglial lysosome of wild-type mice suggested that TRPV4 may regulate lysosomal function. To test this possibility, we conducted pharmacological studies using primary cultures of microglia, and found that treatment with GSK1016790A, a TRPV4 activator, increased cell surface LAMP1, suggesting that TRPV4 activation enhances lysosomal exocytosis in microglia. These results suggest that microglia promote glial scar formation by lysosomal exocytosis via TRPV4 activation.

1-P-021

Microglial Ca^{2+} signals in Alexander disease model

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Alexander disease (AxD) is an intractable neurodegenerative disorder caused by *GFAP* mutations. AxD is a primary astrocyte disease with the pathological hallmark of Rosenthal fibers within astrocytes. AxD astrocytes show several abnormal phenotypes. Our previous study has shown that AxD astrocytes in the model mice show aberrant Ca^{2+} signal that was a cause of etiology of AxD. In addition, we recently found that microglia in AxD mice also exhibited aberrant Ca^{2+} signals. Using Iba1-GCaMP6f-hGFAP mice, an AxD model mice for microglial Ca^{2+} imaging, we studied microglial Ca^{2+} signals with 2 photon microscopy. We found that microglial Ca^{2+} signals were dramatically enhanced in AxD mice with more frequent Ca^{2+} signals in both the processes and cell bodies. Such increases in Ca^{2+} signals were inhibited by $P2Y_{12}R$ antagonist but not by TTX, suggesting that these enhancement should be independent of neuronal activity, but dependent on extracellular ATP-mediated signals. Thus, we think that these microglial abnormal Ca^{2+} signals would be caused by aberrant Ca^{2+} signals in astrocytes. In addition, we already showed that microglia play a protective role in AxD pathology, indicating an importance of microglia-astrocytes communication. Furthermore, to explore how these aberrant AxD microglial Ca^{2+} signals are related to AxD pathology, we performed dual Ca^{2+} imaging of astrocytes and microglia in AxD model in combination with genetic expression profiling by transcriptome analysis. This approach of the research would be critical for understanding the molecular mechanism of which microglia are associated with AxD pathogenesis.

1-P-022

Elucidation of the mechanism of substantia nigra astroglial cell activation by metabolic reactive oxygen species

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Reactive oxygen species (ROS) are implicated in the modulation of diverse processes including glial activation. To evaluate the effects of metabolic ROS produced by mitochondria on astrocyte activation, we created transgenic mice expressing a phosphorylation-defective mutant of succinate dehydrogenase A in astrocytes (aSDHA^{Y215F}). Astrocytes in substantia nigra of in male aSDHA^{Y215F} mice produced three times more ROS than those in control mice, and increased the levels of GFAP expression. On the other hands, the number of TH-positive neurons was significantly reduced. We identified several novel secretion factors from the aSDHA^{Y215F} mice as an inducer of neuronal apoptosis. These results suggest that mitochondrial ROS may regulate dopaminergic neuronal death in substantia nigra through modulation of astrocyte activation. Exact molecular targets for mitochondrial ROS will be discussed.

1-P-023

Effects of CSF1R inhibitor PLX3397 on reinforcement learning ability

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PLX3397 is an orally administrable CSF1 receptor inhibitor that is expected to be a therapeutic agent for cell tumors such as tendon synovial giant cell tumors. Administration of high doses of PLX3397 is known to eliminate microglia selectively and may affect neuronal functions via the loss of microglia. The elimination of microglia has been known to affect exploratory tasks involving aversive stimuli. However, the effect of the microglial elimination on the ability of reward-based reinforcement learning has been unknown. In this study, C57BL6 mice were administrated with PLX3397 for three weeks and were tested with a 5-armed bandit task (5-ABT). 5-ABT is an exploratory operant conditioning task in which each of the five choices has a different reward probability. The task contains three subtasks: one in which all choices had a reward probability of 30% (ALL), a subtask in which only one choice had a reward probability of 50% and the rest had a reward probability of 0% (BIT), and a subtask in which the correct choice in BIT was reversed and four options had a reward probability of 30% (REV). As a result, there was no significant difference in the entropy of choice (exploration pattern) in the subtask ALL between the PLX3397-treated group and the control group. There was no significant difference between the groups in the number of steps required for the BIT and REV subtasks to exceed 50% correct. We also estimated the learning rates of the mice by fitting the behavioral sequences to a Q-learning model and found no significant differences between the groups. These results suggest that microglial elimination has no significant impact on reinforcement learning ability.

1-P-025

Functional regulation targeting nicotinic acetylcholine receptors using iPS cell-derived microglia

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Microglia are tissue-resident macrophages located in brain parenchyma and play key roles not only in brain immunity but also interact with neurons to contribute to neurogenesis, axonal growth, and synaptic refinement. The origin of microglia has been shown to be primitive macrophages that arise in the yolk sac during embryonic hematopoiesis colonize the developing brain prior to the establishment of the blood-brain-barrier. Alzheimer's disease (AD) is the progressive neurodegenerative disease characterized by the accumulation of amyloid-β (Aβ). Clearance by Aβ phagocytosis has been suggested as a function of microglia in AD brains. We here prepare the mouse induced pluripotent stem cells (iPSCs)-derived primitive macrophage (iMacs) as a cellular model of microglia that recapitulates their cellular origin and examined the functional regulation targeting on nicotinic acetylcholine receptors (nAChRs). We further examined cellular derivation into the brain by the peripheral injection of iMacs for the future cell therapeutic strategy for AD. Stimulation of α7 nAChR on iMacs by galantamine promoted Aβ phagocytosis. iMacs were further transplanted via tail vein after endogenous microglia depletion with PLX3397, an inhibitor of colony stimulating factor 1 receptor, and X-ray irradiation. As a result, a part of iMacs was found in the brain. Analysis of microglial function using a cell model that takes into account the cellular origin may contribute to the development of therapeutic strategies for AD.

1-P-026

Deep learning-based modeling of stress-induced epigenomic changes in microglia and prediction of transcriptional networks

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Stress due to adverse and demanding conditions causes emotional disturbances and increases the risk of mental illness such as depression. Chronic stress, including repeated social defeat stress, activates prefrontal microglia to induce depressive-like behavior in mice. With ChIP-seq analyses, we found that long-term epigenomic changes accompany this microglial activation. However, cis-regulatory interactions among enhancers and promoters for the epigenomic changes remain unsolved. Here we trained a deep learning-based model with our microglial ChIP-seq data segmented into approximately 130k base-pair segments with high accuracy ($r = 0.654$). *In silico* mutagenesis in this model revealed genomic regions responsible for stress-induced epigenomic changes at single-nucleotide resolution. Notably, the predicted genomic regions matched nucleosome-free regions predicted from microglial ATAC-seq data that had not been used to train the model. We identified transcription factor binding motifs enriched in the predicted genomic regions and pairs among them in proximity. Our deep learning-based epigenomic analyses offer a novel method to predict chromatin interactions at single-nucleotide resolution even with limited sample sizes and pave the way for elucidating transcriptional networks underlying health and diseases, including stress and depression.

1-P-027

NMDA-induced activation of the CaMKII-RhoA-Rho-kinase pathway regulates aversive learning

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Glutamate induces Ca^{2+} influx in neurons through NMDA receptors (NMDARs) and consequently activates protein kinases, including CaMKII, which plays critical roles in postsynaptic functions and learning. However, how CaMKII regulates learning remains largely unknown. Here, we show that NMDA-induced activation of the CaMKII-RhoA-Rho-kinase pathway regulates aversive learning through Shank3 phosphorylation. We performed phosphoproteomics to identify CaMKII substrates and found that CaMKII phosphorylated ArhGEF2 (RhoGEF) and stimulated its RhoGEF activity downstream of NMDAR. Aversive stimuli induced CaMKII-mediated ArhGEF2 phosphorylation and Rho-kinase/ROCK activation in the nucleus accumbens (NAc). Inhibition of Rho-kinase in dopamine D2 receptor (D2R)-expressing medium spiny neurons (MSNs) in the NAc attenuated aversive learning. We also found that Rho-kinase phosphorylated Shank3 and increased its interaction with NMDAR and AMPA receptors. Manipulation of Shank3 in D2R-MSNs regulated dendritic spine morphology and aversive learning in a phosphorylation-dependent manner. These results demonstrated that NMDA-induced phosphorylation of Shank3 via the CaMKII-Rho-kinase pathway regulates aversive learning.

1-P-028

Anti-fatigue effects of 5-Aminolevulinic Acid in a mice Chronic Fatigue Model

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[Introduction] 5-aminolevulinic acid (ALA) is a precursor of heme and is involved in mitochondrial activation. It has been suggested that mitochondrial dysfunction in the brain is associated with chronic fatigue. To clarify the anti-fatigue effect of ALA, we used the established chronic fatigue model mice and examined the effects of ALA on fatigue and noradrenaline (NA) level in the frontal cortex (FCX).

[Methods] Female C57BL/6N mice were orally given ALA hydrochloride or DW for 8 weeks. The fatigue model mice were developed by housing them in a cage filled with water to a height of 1.5 cm for 4 days. The fatigue was evaluated using the running distance in the treadmill test. After the test, their brains were quickly dissected and NA in the FCX were quantified by HPLC.

[Results and Discussion] The running distance in the treadmill test were significantly reduced in the fatigue group. In addition, the running distance in the ALA-treated fatigue group was significantly increased as compared with DW-treated fatigue group. In the fatigue group, NA contents in the FCX were significantly decreased and ALA treatment canceled it. These results suggested that chronic ALA treatment produced the anti-fatigue effect and the protective effects on NA neurons in the FCX which may be involved in the mechanisms of the effect of ALA.

1-P-029

Melanocortin 3/4 receptor in the dorsal raphe nucleus regulates maternal care in lactating mice

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Maternal care is indispensable for survival of neonates in mammals. Lactating females consume a large amount of energy for nurturing their pups by lactation. Management of energy expenditure through lactation is important for survival of themselves and pups. We previously reported that the orexigenic neuropeptide neuropeptide Y in the dorsal raphe nucleus (DRN) regulated maternal care, depending on food intakes of lactating females. In the present study, we investigated the neuronal mechanism for regulating maternal care by melanocortin 3 and 4 receptors (MC 3/4R), which was regulated by the anorexigenic neuropeptide alpha-melanocyte-stimulating hormone (α-MSH) and the orexigenic neuropeptide agouti-related peptide (AgRP) in an opposing manner. Neuronal processes immunoreactive to adrenocorticotropic hormone, which was a precursor of α-MSH, or AgRP, were distributed in the DRN. Furthermore, the pre-synaptic marker synaptophysin was co-localized with ACTH or AgRP in the DRN. We next investigated how the MC3/4R antagonist SHU 9119 affected maternal care. Injection of 100 pmol SHU 9119 into the DRN prevented maternal care in fed dams. Additionally, we examined whether the agonist melanotan II could affect maternal care following fasting for 8 h. Fasting for 8 h abolished maternal care in lactating females. But injection of 100 pmol melanotan II into the DRN partially recovered maternal care. These results indicate that MC3/4R signaling in the DRN regulates maternal care depending on feed intake in lactating mice.

1-P-030

Enhanced sensitivity of epileptic seizure by deficit of hyperpolarization activated cyclic nucleotide-gated (HCN) channel 1

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Hyperpolarization activated cyclic nucleotide-gated (HCN) channels underlie hyperpolarization-activated current (I_h) generation, regulating spontaneous rhythm and neural oscillation. HCN1 channels are abundantly expressed in the cerebral cortex, hippocampus and brain stem and are suggested to be involved in the initiation and propagation of spontaneous generalized seizure, however, the functional mechanism is still unknown. In this study, to clarify the role of HCN1 channel in induction of epileptic seizure, we performed the chemically- and electrically-induced seizure tests using *Hcn1* knock-out (*Hcn1*-KO) rats. Pilocarpine and 4-aminopyridine produced significantly higher seizure induction in *Hcn1*-KO rats than in control (F344) rats. *Hcn1*-KO rats also showed higher sensitivity to electrical shock-induced seizures. In addition, we performed the immunohistochemical analysis of c-Fos expression following electrical shock-induced seizures. *Hcn1*-KO rats showed a significantly higher Fos expression than control rats in the cerebral cortex and amygdala. These results suggest that HCN1 channels play a crucial role in controlling the susceptibility to epileptic seizure, implying that hyperactivation of the cerebral cortex and amygdala is involved in the enhancement of seizure susceptibility due to loss of HCN1 channel.

1-P-031

What methods are physiologically or pharmacologically effective for attracting female mice?

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We've found the presence of attractive or unattractive male mice among four littermate male mice by behavior-based measurement with video camera tracking system. This trend of preference disappeared by hiding male mice with four-layered air-permeable filter. Furthermore, genetically blind female mice showed completely different trend of preference against the same male mice set, indicating that appearance may be one of major factors of male attractiveness.

To increase or decrease male attractiveness, we've tried several approaches.

1) One male mouse and one female mouse are bred as a mating pair for three months to and see whether the female develops a preference for the male.

2) When a female mouse is meeting with an unattractive male mouse, isoproterenol is injected into the female mouse just before the meeting to see if she will mistake it for a crush on the male mouse.

3) Optogenetic stimulation of dopaminergic nerves that project to the nucleus accumbens only when approaching an unattractive male mouse during the male preference test.

4) Unattractive male mice were given daily subcutaneous injections of testosterone for a week to see if their attractiveness would increase.

In these approaches, only testosterone injections were able to increase the attractiveness of the unattractive male mice. It is possible that testosterone directly affected the brain, influencing attitude and pheromone release, since the changes were seen within a week. We hope to elucidate the mechanism in the future.

1-P-032

Central effects of xenin in nesfatin-1 neurons in rat

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INTRODUCTION

Xenin is a 25-amino acid peptide identified from human gastric mucosa. Centrally and peripherally administered xenin decreases food intake in rodents. A previous study showed that xenin-induced anorexia was mediated by the neurotensin and CRF receptors. However, the central mechanism of xenin-induced anorexia has been unclear yet. Nesfatin-1/NucB2 (nesfatin-1) is newly identified as an anorexia neuropeptide. We examined the central effects of xenin on food intake, water intake and Nesfatin-1 like immunoreactivity (nesfatin-1-LI) neurons in rat.

METHODS

After intracerebroventricular (icv) administration of xenin, we examined the Fos like immunoreactivity (Fos-LI) and nesfatin-1-LI expression by immunohistochemistry in rat brain. We also measured food and water intake, after icv administration of xenin with pretreatment of nesfatin-1 antisense.

RESULTS

Fos-LI expressed in the supraoptic nucleus (SON), paraventricular nucleus (PVN), arcuate nucleus, central amygdaloid nucleus, area postrema, and nucleus of the solitary tract after icv administration of xenin. Icv administered xenin caused significant increases the number of Fos-LI in expressing nesfatin-1-LI neurons in the SON and PVN. Furthermore, icv administration of xenin significantly decreased food intake. This xenin-induced food suppression was significantly attenuated by pretreatment with icv administration of antisense nesfatin-1.

CONCLUSION

These results indicate that nesfatin-1 may play an important role in xenin-induced food suppression in rats.

1-P-033

Generation and analysis of voltage-gated calcium channel β_4 subunit conditional knock-out mice

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The number of patients suffering from psychiatric disorders is extremely high worldwide. It has been identified that genes encoding voltage-gated calcium channels (VGCCs) subunits are associated with psychiatric disorders such as schizophrenia. The functions of VGCCs have been elucidated at the molecular level, whereas their roles in higher brain functions are largely unknown. In this study, we generated a forebrain glutamatergic neurons-specific VGCC β_4 subunit knock-out mice and conducted a comprehensive behavioral test. β_4 conditional knock-out mice showed altered sociability in three-chamber social interaction test. These knock-out mice also exhibited increased startle response. Therefore, this VGCC β_4 subunit conditional knock-out mouse may become a useful model for studying the psychiatric disorders related to sociability or auditory function.

1-P-034

Boredom turns aversive stimuli into rewards

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We get bored when we have nothing to do. The psychological definition of boredom is the aversive state of wanting, but being unable, to engage in satisfying activity. To avoid feeling boredom, humans left in an empty room actively receive aversive shocks. Here, we found that mice also received aversive air puff stimuli more frequently when they were placed in an empty cage than in an enriched cage with toys. Some mice exhibited an addiction-like state in which they received air puffs more than once per second. This self-stimulation increased and decreased by activation and inactivation of the insular cortex, which is related with boredom in humans, respectively. Next, we recorded neural activities from the insular cortex. Using support vector machine, we can predict the timing of self-stimulation with spiking activity of insular cortical neurons. To investigate the neural mechanism of receiving aversive air puff stimuli, we recorded dopamine signals of the ventrolateral striatum that expressed a dopamine sensor. Dopamine levels increased before receiving air puffs actively, while decreasing after receiving air puffs. Finally, an μ-opioid receptor antagonist decreased self-stimulation including the addiction-like behavior. This study suggests that the state of boredom converts aversive stimuli to rewards and will provide clinical insights into addiction and self-injury.

1-P-035

A cannabinoid CB_1 receptor knockout mouse demonstrates autism spectrum disorder-like behaviors

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Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental disability that demonstrates impaired social interactions, social communication deficits, and restrictive/repetitive behaviors. The endocannabinoid (eCB) system in the brain is a major regulator of synaptic plasticity and neuromodulation. It is reported that the eCB system have been altered in children with ASD and some animal models of ASD. To determine the causal role of the eCB system in the ASD, we have investigated the relationship between declines of the eCB system and the ASD-like symptoms, using the cannabinoid CB_1 receptor knockout (CB1KO) mice. We found that male CB1KO mice demonstrated reduced sociability (3-chambered social approach task), elevated repetitive grooming behaviors (hole-board test) and deficits in short-term memory (Y-maze test). Moreover, the CB1KO mice also showed emotional instabilities (elevated plus-maze test and hole-board test). The serum progranulin, which is lower levels in patients of ASD, was significantly decreased in CB1KO mice. These findings suggested that CB1KO mice showed behavioral phenotypes including social deficits, which have face validity as an animal model of ASD. Therefore, the CB1KO mice will be a valuable tool for the exploration of pathological mechanisms and development of novel therapeutics in the ASD.

1-P-036

What symptoms are associated with aggressive biting behavior of isolation-reared mice?

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[Background and purpose] The Aggressive Response Meter (ARM) can be used to evaluate the efficacy of drugs that are effective in treating aggressive biting behavior of this isolated-reared mouse (Igarashi et al., Brain Res., 2021). However, the pathology caused by isolation-rearing stress is complex, and it was not clear what symptoms this aggression was associated with. In this study, we conducted multiple behavioral tests on isolation-reared mice and investigated the correlation of each experimental parameter for each individual.

[Method] Male and female ddY strain mice were individually bred from 3 weeks after birth, and biting behavior was measured at 10 weeks of age. In addition, open field test (OFT), a resident-intruder (R-I) test, and elevated plus maze (EPM) were conducted.

[Results] When the correlation coefficient of Pearson was examined for the bite strength measured by ARM and the number of aggression behaviors in the R-I test for isolated male mice, it showed a negative value ($r = -0.40$, $p = 0.26$), no aggressive behavior was observed in the R-I test in female mice. In addition, when the relationship between biting intensity and time in central zone was investigated in OFT, a weak positive correlation was shown in male mice ($r = 0.23$, $p = 0.51$), and a positive correlation was shown in female mice ($r = 0.63$, $p = 0.049$). Examination of biting intensity and open arm residence time in EPM showed a negative correlation in male mice ($r = -0.61$, $p = 0.06$) and a weak positive correlation in female mice ($r = 0.20$, $p = 0.58$).

[Discussion] The bite strength measured by ARM is considered to reflect some pathological aggression (Kuchiiwa and Kuchiiwa, J. Neurosci. Methods, 2014). From the above results, it is considered that the aggressive biting behavior measured by ARM indicates some features of mental symptoms in each sex.

1-P-037

Hexagonal columnar animal cage: Mechanism of social adaptation by controlling inter-individual communication in mice

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To be health, it is important to adapt to society in which everything communicates with each other. However, the mechanism of social adaptation is poorly understood due to the lack of established methodologies that control intra-individual communication. To solve this problem, we have developed the hexagonal columnar animal cage that can control communication within individual mice by using removal partitions. In the experiment, male ICR-, ddY-BALB/c-, and C57BL/6J-strains of mice were used to evaluate the relationship between contact conditions and depression in tail suspension test. In the group in which only three mice of the same strain were contacted, the depression level of ICR mice was not different even when the contact conditions were changed. The depression levels of ddY mice were exacerbated when separated from each other by using partitions with or without punched holes. The depression levels of BALB/c mice were exacerbated only when isolated by using partitions without punched holes. On the other hand, the partition with punched holes reduced the depression levels in C57BL/6J mice. These results indicate that means of stress-inducible social communication is different between mice strain. Our hexagonal columnar animal cage revealed that there are diverse communication styles for adapting to society.

1-P-038

Effects of N-terminal fragment of canstatin on left ventricular hypertrophy in a rat model of angiotensin II-induced hypertension

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Canstatin, a cleaved fragment of type IV collagen α2 chain, is highly expressed in myocardium of normal rats. We previously demonstrated that full length canstatin exerts an anti-hypertrophic effect in several rat cardiac disease models. However, the active sites of canstatin have not been determined. In this study, we explored the active sites of canstatin, which could exhibit protective effects against angiotensin II (Ang II)-induced cardiac hypertrophy. Full length and N-terminal (1-82 amino acids: N-canstatin) but not central (83-149 amino acids) or C-terminal (150-220 amino acids) fragment of canstatin inhibited Ang II-induced hypertrophy in neonatal rat cardiomyocytes. In a rat model of Ang II-induced hypertension, N-canstatin did not affect an increase in systolic blood pressure, while it showed a tendency to inhibit an increase in heart weight/tibia length ratio and significantly suppressed cardiomyocyte hypertrophy. N-canstatin significantly inhibited myocardial fibrosis and tended to suppress mRNA expression of type I collagen in left ventricles. In the present study, we for the first time demonstrate that N-terminal fragment of canstatin has protective effects against hypertrophic cardiac remodeling.

1-P-039

Expression of arresten and its role in the heart of rats with angiotensin II-induced hypertension

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Type IV collagen is a major component of basement membrane around cardiomyocytes. Arresten, a C-terminal fragment of type IV collagen α1 chain, is highly expressed in heart tissue of normal rats. However, expression of arresten and its role in cardiac hypertrophy have not been determined. In the present study, we investigated the expression of arresten in hypertrophied myocardium of rats with angiotensin II (Ang II)-induced hypertension. In addition, the effect of arresten on Ang II-induced hypertrophy in neonatal rat cardiomyocytes (NRCMs) was examined. We confirmed that an infusion of Ang II (500 ng/kg/min, 4 weeks) induced hypertension and subsequent left ventricular hypertrophy in rats. The expression of arresten in hypertrophied left ventricles tended to be increased. Recombinant rat arresten (100 ng/ml) significantly enhanced Ang II (1 μM, 48 h)-induced hypertrophy in NRCMs. In the present study, we for the first time demonstrate that arresten is upregulated in left ventricles of rats with Ang II-induced hypertension, which might contribute to cardiac hypertrophy.

1-P-040

Improvement effect of Eucommia ulmoides oliver derived geniposidic acid on hypoxia-induced pulmonary arterial hypertension

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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. We recently showed that Eucommia ulmoides oliver leaf extract (ELE) improve right ventricular systolic pressure (RVSP) and pulmonary vessel muscularization. In the present study, we investigated the effects of geniposidic acid, a major component of ELE, on hypoxia-induced PAH in mice. 8-weeks-old male C57BL/6J mice were orally administered geniposidic acid (～1 mg/kg, ～5 mg/kg) during exposure to hypoxia for 4 weeks. Geniposidic acid significantly suppressed the elevation of RVSP in hypoxia-induced PAH mice. In addition, hypoxia-induced pulmonary arterial muscularization was tended to be attenuated in geniposidic acid-treated mice. In human pulmonary artery smooth muscle cells (HPASMC), endothelin-1-induced increase of intracellular Ca^{2+} was attenuated by geniposidic acid (200 μM). Furthermore, geniposidic acid (50 – 200 μM) increased the maximal respiration in HPASMC. Our findings suggest that geniposidic acid may effectively improve the development of hypoxia-induced PAH by preventing the vascular remodeling and mitochondrial dysfunction of pulmonary artery.

1-P-042

Disruption of Actin Dynamics Regulated by Rho Effector mDia1 Attenuates Pressure Overload-Induced Hypertrophic Responses in Cardiac Ventricle and Exacerbates Dysfunction.

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Cardiac hypertrophy is a compensatory response to pressure overload, leading to heart failure. Recent studies have demonstrated crucial roles of Rho and its downstream proteins in cardiac hypertrophic responses. However, the detail mechanisms how Rho signaling controls hypertrophic responses remain incompletely understood. Here, we show that mammalian homolog of Drosophila diaphanous (mDia) 1, a Rho-effector molecule, plays pivotal roles in pressure overload-induced ventricular hypertrophy. mDia1-knockout (mDia1KO) mice subjected to transverse aortic constriction (TAC) exhibited attenuated cardiac hypertrophic responses in the early phase and poor survival in the chronic phase. Microarray analysis revealed that mDia1 was involved in the induction of hypertrophy related genes targeted by the serum response factor (SRF) pathway, e.g. immediate early genes, in pressure overloaded hearts. Loss of mDia1 attenuated the activation of focal adhesion kinase and extracellular signal-regulated kinase in both TAC-operated mice hearts and mechanical stretched neonatal rat ventricular cardiomyocytes. Furthermore, the increase in the F/G-actin ratio induced by TAC was suppressed in mDia1KO hearts, thereby resulted in a decrease in the nuclear accumulation of myocardin-related transcription factors and SRF. Our data demonstrate that mDia1 coordinates signal transduction triggered by pressure overload through the regulation of actin homeostasis, which is required for proper cardiac responses to pathological stimuli.

1-P-043

Phospholamban is degraded by parkin-mediated ubiquitination in failing heart

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Both parkin (an E3 ubiquitin ligase) and PINK1 (a PTEN-induced putative kinase 1), master regulators of mitophagy, undergoes the degradation of excess mitochondria to maintain cellular homeostasis. Although parkin and PINK1 are expressed in the heart, its pathophysiological role remains unknown. Here, we found that phosphorylation level of parkin at serine 65 was up-regulated, regardless of decreased level of total parkin protein in cardiomyocytes excised from dilated cardiomyopathy (DCM) mice compared to control mice. Also, both parkin and PINK1 bound to phospholamban (PLN), a potent inhibitor of sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA2a), in cardiomyocytes excised from DCM mice tighter than control mice. Furthermore, we observed that the expression level of PLN was decreased in *parkin* overexpressing HEK293 cells, whereas knockdown of *parkin* increased PLN expression level in HEK293 cells. These data indicate that PLN may be one of the substrates for parkin, and is degraded via parkin-mediated polyubiquitination in failing hearts. Future work is necessary to determine whether parkin can regulate cardiac function in different stages of DCM especially through its role in regulating PLN levels.

1-P-044

Cardiac-specific overexpression of HDAC6$^{H216A, H611A}$ can prevent the development of heart failure attributable to pressure overload in mice

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Histone deacetylase (HDAC) 6 is known to deacetylate amino acid lysine in alpha-tubulin, a component of microtubules, at amino acid position 40. However, the functional role of HDAC6 in the progression of cardiac disease conditions such as pressure-overloaded hypertrophy remain uncertain. The involvement of HDAC6 in cardiac disease was examined using transgenic (TG) mice expressing human dominant-negative HDAC6 (HDAC6H216A, H611A, 4-fold increase) specifically in the heart. Overexpression of HDAC6H216A, H611A significantly increased acetylated alpha-tubulin in mouse hearts, suggesting that HDAC6 can regulate cardiac tubulin deacetylation. Neither histological alteration in the myocardium nor alteration of the cardiac function determined by echocardiography was observed in the HDAC6H216A, H611A TG mice 1 year of age or older. To analyze the role of HDAC6 and acetylated tubulin in disease conditions, we examined the role of HDAC6 in pressure-overloaded hypertrophy generated by transverse aortic constriction (TAC) surgery. A reduction in the shortening fraction was detected in the NTG mouse hearts 4 weeks after TAC surgery while a sustained shortening fraction was observed in the TAC HDAC6H216A, H611A TG mouse hearts, suggesting that inactivation of HADC6 with concomitant enhancement in acetylated tubulin can prevent cardiac disease in pressure-overloaded hypertrophy.

1-P-045

Specific degrader of hematopoietic prostaglandin D synthase prevented the progression of dilated cardiomyopathy in Duchenne muscular dystrophy.

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Duchenne muscular dystrophy (DMD) is a severe muscle disease caused by mutations in the dystrophin gene. We have found that hematopoietic prostaglandin (PG) D synthase (HPGDS) was induced in grouped necrotic muscle fibers in DMD patients and also in model mice (*mdx*). DMD also affects cardiac muscle and cardiac failures are the most common causes of death in DMD patients. In this study, we developed a novel specific degrader for HPGDS protein composed of the HPGDS inhibitor and E3 ligase ligand and investigated beneficial role of HPGDS degradation to cardiac function and morphology in heart of *mdx* mice.

Dilated cardiomyopathy was induced in *mdx* mice by thyroid hormone [3,3‘,5-triiodo-L-thyronine (T3)]. T3 was daily injected subcutaneously in *mdx* mice for 2 weeks. HPGDS specific degrader was daily administered in T3-treated *mdx* mice. Serum cardiac troponin I and mRNAs of inflammatory cytokines in heart were measured. The severity of cardiomyopathy or fibrosis was histochemically evaluated.

mRNA of HPGDS, cyclooxygenases and prostanoid DP receptors were significantly upregulated after T3-treatment. In HPGDS specific degrader administrated T3-treated *mdx*, the hypertrophy of heart was significantly reduced compared to the vehicle-treated. HPGDS specific degrader treatment lowered the serum cardiac troponin I, prevented the expression of inflammatory cytokines, and decelerated the progression of fibrosis.

PGD_2 inhibition by HPGDS degradation prevents the progression of dilated cardiomyopathy in DMD mice.

1-P-046

Therapeutic effects of voluntary wheel running on cancer cachexia-induced cardiac dysfunction: Approach from the perspective of Cardio-oncology

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Recently, the interdisciplinary field of cardio-oncology has emerged to study the mechanisms of cardiac dysfunction associated with cancer treatment and how to prevent it. In this study, we investigated possible therapeutic effects of voluntary wheel running (VWR) on cardiac dysfunction observed in a cancer-cachexia model mice established by our laboratory. VWR starting from 2 to 6 wks after implantation of tumor cells significantly suppressed the loss of heart and skeletal muscle weight as well as general symptoms of cachexia. Moreover, left ventricular ejection fraction significantly increased in cachexia group with VWR, compared to those without VWR. Microarray analysis revealed that the expression of gene “X”, which is an enzyme belonging to E3 ubiquitin ligase family and has not been reported to be related to skeletal muscular atrophy, increased in the myocardium of cachexia mice, and that this increase was suppressed by VWR. These results suggest that the mechanism of myocardial impairment may be different from that of skeletal muscle atrophy, and that VWR may improve not only cachexia symptoms but also cachexia-induced cardiac dysfunction. In addition, the gene “X” may be one of key factors which are associated with myocardial atrophy and cardiac dysfunction on cancer cachexia. The pathway mediated by the gene “X” is currently being further analyzed.

1-P-047

SIRT1 protects the cardiomyocyte from doxorubicin-induced injury by mediating DNA damage response via deacetylation of histone H2AX.

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Introduction: We recently found that deletion of SIRT1, a histone deacetylase, in the cardiomyocyte worsens doxorubicin (DOX)-induced cardiac injury in mice. However, its molecular mechanism remains unclear. We hypothesized that SIRT1 protects cardiomyocytes from DOX-induced injury by mediating DNA damage response (DDR) via deacetylation of H2AX, a critical mediator of DDR.

Methods and Results: In H9c2 cardiomyocytes, treatment with Dox increased cleaved caspase-3 level, which was enhanced by knockdown (KD) of SIRT1. The comet assay showed that SIRT1 KD also increased the degree of DNA damage induced by Dox. Immunoblotting revealed that treatment of cells with Dox increased levels of Ser139 phosphorylation of H2AX, indicating the DDR by Dox. SIRT1 KD abolished this response. Immunostaining showed that acetyl-Lys5 H2AX level was increased by SIRT1 KD and reduced by expression of wild-type SIRT1. In COS7 cells, a mutant in which Lys5 was substituted to glutamine (K5Q H2AX), a mimic of acetylated Lys5, showed attenuated Ser139 phosphorylation of H2AX by DOX. In H9c2 cells, DOX-induced cleavage of caspase-3 was enhanced in cells expressing K5Q H2AX as well as S139A H2AX, that cannot be phosphorylated at Ser139, compared with cells expressing WT H2AX.

Conclusions: Aberrant Lys5 acetylation of H2AX via SIRT1 suppression interferes Ser139 phosphorylation, leading to accumulation of damaged DNA and apoptotic cell death. Such regulation may underlie protection by SIRT1 against DOX-induced cardiotoxicity.

1-P-048

Assessment of drug-induced contractility by Electric Cell-substrate Impedance Sensing in hiPSC-CMs

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Assessment of drug-induced cardiotoxicity plays an important role in drug development. Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have been widely used as a platform to evaluate cardiac toxicity/safety during drug development. Multi-electrode array (MEA) system with iPSC-CMs has been one of the standard assay systems to predict drug-induced proarrhythmic risk. In addition to MEA system, contractility is other key issues to prevent cardiotoxicity at later stages for drug development. Among various in vitro approaches, we focused on the electric cell-substrate impedance sensing system (ECIS) to monitor contractility. Although ECIS are expected for high-throughput screening, correlation between ECIS and contractility has not been fully elucidated. In the present study, we compared with waveform detected sequentially by ECIS and imaging-based cell motion system (CMI) using hiPSC-CMs (iCell CM, CDI). We found that several parameters, such as inflection point, beat rate, contraction rate, were correlated between ECIS and CMI. In addition, correlation with these parameters were confirmed in the treatment of isoproterenol and verapamil. These data suggest that contractility can be assessed by both impedance and imaging in iPSC-CMs. We are now planning to evaluate contractility using more compounds, such as anti-cancer agents, in iPSC-CMs.

1-P-049

Chronic toxicity assessment of BCR-ABL tyrosine kinase inhibitors using human-induced pluripotent stem cell-derived cardiomyocytes

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Tyrosine kinase inhibitors (TKIs) have contributed to the improvement of the survival of patients with chronic myelogenous leukemia (CML). Growing evidence suggest that cancer therapy-related cardiac dysfunction has become important as the most undesirable side effects of chemotherapy. BCR-ABL TKIs, such as nilotinib and imatinib, has been reported to have a risk of QT prolongation associated with Torsades des Pointes and cardiac failure. We have previously reported that nilotinib caused QT prolongation and early afterdepolarization (EAD) using human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). However, chronic cardiotoxicity of BCR-ABL TKIs has not been fully understood. In this study, we evaluated the chronic toxicity of imatinib and nilotinib using hiPSC-CMs. We used iCell Cardiomyocyte 2.0 (Cellular Dynamics International). To assess whether imatinib and nilotinib induced chronic cardiotoxicity, we investigated the QT interval recorded by multi-electrode array system (MED64, Alpha MED Scientific) and contractility velocity recorded by motion vector analysis (SI8000, Sony). We found that the imatinib decreased contraction velocity for long-term treatment. In addition, long-term treatment with nilotinib caused QT prolongation and decrease in contraction velocity in a concentration-dependent manner. These results suggest that hiPSC-CMs can be the useful tool for chronic cardiotoxicity assessment. We are planning to evaluate other types of BCR-ABL TKIs with hiPSC-CMs.

1-P-050

Effects of cigarette smoke extract (CSE) on the contraction, spontaneous beating rate and intracellular Ca^{2+} dynamics in adult and neonatal cardiomyocytes

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Smoking is a known risk factor of ischemic heart diseases via various toxic effects on several tissues, including lung and cardiovascular systems. However, the direct effects of smoking substances on the cardiomyocyte function and its cellular mechanism have not been fully clarified. In the present study, we examined the effects of CSE on the contractile function and intracellular Ca^{2+} transients using freshly isolated adult and cultured neonatal cardiomyocytes. These parameters were analyzed using the Cell Motion Imaging System (Sony SI8000). Application of 1% CSE significantly increased the spontaneous beating rate of cultured neonatal cardiomyocytes. On the other hand, the same treatment decreased the cell shortening of electrically stimulated adult cardiomyocytes. Intracellular Ca^{2+} transients obtained from Fluo-8-loaded cardiomyocytes showed that 1% CSE induced massive increase in diastolic Ca^{2+} levels, little change in systolic levels, and as a result, significant decrease in Ca^{2+} transient amplitude. These results suggest that CSE weaken the contractile function of cardiomyocytes by altering intracellular Ca^{2+} dynamics, while increasing in the beating rate. Further cellular mechanisms, such as the possibility of Ca^{2+} leak from the sarcoplasmic reticulum and change in the myofilament Ca^{2+} sensitivity, would be discussed.

1-P-051

The importance of sympathetic innervation for the postnatal heart development

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The sympathetic nervous system is important for the adult heart to adapt to the changes in environmental stresses. Besides that, it has been suggested that sympathetic innervation affects postnatal development of the heart. Several lines of evidence have indicated that sympathetic innervation changes the modes of cardiomyocyte growth from proliferation to hypertrophy and the membrane excitability by altering ion channel activities. However, most of these findings were obtained from the in vitro co-culture of neonatal cardiomyocytes and sympathetic neurons. Therefore, whether it is also the case in vivo remains largely unknown. In this study, we have analyzed the effect of chemical sympathetic denervation on the postnatal murine heart development before weaning (at postnatal day (P) ~20) by treating newborn pups (P0-1) with 6-hydroxydopamine (6-OHDA). Immunofluorescence staining of tyrosine hydroxylase, a marker of sympathetic neurons, demonstrated that the sympathetic neurons were almost completely eliminated in P7 to 21 mice hearts by 6-OHDA treatment. 6-OHDA treatment significantly reduced left ventricular contractility compared with the control at P21. However, neither L type calcium channel activity nor calcium transients evoked by field stimulation were unchanged in the isolated cardiomyocytes from P21 mice hearts. Fluorescent imaging of T-tubule structures demonstrated that cardiomyocytes from 6-OHDA-treated mice hearts have reduced regularity of T-tubule structure. Furthermore, the protein abundance of alpha-SMA, a vascular smooth muscle cell marker, was significantly reduced in 6-OHDA-treated mice hearts at P21. These results suggest that sympathetic innervation is likely to play critical roles in the postnatal maturation of cardiomyocytes and coronary circulation.

1-P-052

The effects of a Src inhibitor on allergic airway inflammation and its exacerbation of mice induced by house dust mite and poly(I:C)

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The exacerbation of asthma is often caused by airway infections, resulting the corticosteroid resistance and the difficulty to control asthma symptoms. Recently, it was reported that Src was involved in inflammatory responses. We also reported that dasatinib (DAS), a Src inhibitor, suppressed corticosteroid insensitive airway inflammation of mice induced by lipopolysaccharide. Thus, we determined effects of DAS on airway inflammation of mice induced by house dust mite (HDM) and poly(I:C).

Mice, sensitized to HDM, were intranasally challenged with HDM once every other day for 11 days, followed by treatment with DAS or fluticasone propionate (FP) twice daily for 3days. Some mice were also exposed to poly(I:C) 2hr after each drug treatment. One day after the last drug treatment, bronchoalveolar lavage fluid (BALF) was collected. The numbers of eosinophils and neutrophils, and the levels of CXCL1, IL-13 and IL-33 in BALF were measured.

DAS suppressed the increases in inflammatory cells and cytokine/chemokine levels induced by HDM alone and HMD + poly(I:C), whereas FP had little effects on these increases induced by HDM + poly(I:C). These results suggested that Src will be one of the important therapeutic target for asthma and its exacerbation.

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1-P-053

Analysis of the role of macrophage senescence in lung fibrosis using macrophage-specific p16 knockout mice

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The involvement of senescent cells in the lungs of idiopathic pulmonary fibrosis has been strongly suggested. In our previous study, we confirmed cellular senescence of macrophages and expression of $p16^{INK4a}$, a key marker of cellular senescence, in the lungs from bleomycin-induced pulmonary fibrosis model mice. However, the role of macrophage senescence in fibrosis has not been clarified. In the present study, we investigated the role of macrophage senescence in lung fibrosis using macrophage-specific p16 knockout (p16 cKO) mice. While bleomycin treatment increased p16 mRNA expression in whole lung tissue from the control mice, such an effect was not observed in the p16 cKO mice. We also examined expression of p16 mRNA in macrophages isolated from the lung and confirmed that the levels were below the detection limit in p16 cKO mice. However, Sirius Red staining revealed no difference in collagen accumulation by bleomycin treatment between lungs from control and p16 cKO mice. In addition, there was no difference in the soluble collagen concentrations, cell numbers or protein concentrations in bronchoalveolar lavage fluid, which are indicators of pulmonary fibrosis. These results suggest that macrophage senescence may have little effect on lung fibrosis.

1-P-054

Effect of NP-1815-PX (a P2X4 receptor antagonist) on the contraction of guinea pig tracheal smooth muscles

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NP-1815-PX is a selective P2X4 receptor antagonist. Administration of a P2X4 receptor antagonist to asthma model mice improves the asthma symptoms, suggesting that P2X4 receptor antagonists may be new therapeutics for asthma. However, the effect of these antagonists on tracheal smooth muscles (TSMs) has not been investigated. This study examined the effect of NP-1815-PX on guinea pig TSM contraction. In epithelium-intact TSMs, NP-1815-PX strongly suppressed ATP-induced contraction, which was suppressed by indomethacin or ONO-8130 (an EP_1 receptor antagonist). NP-1815-PX strongly suppressed U46619 (a TP receptor agonist)- and $PGF_{2\alpha}$-induced epithelium-removed TSM contractions, which were largely inhibited by SQ 29,548 (a TP receptor antagonist). Additionally, NP-1815-PX strongly suppressed the U46619-induced increase in intracellular Ca^{2+} concentration in TP receptor-expressing cells. In contrast, NP-1815-PX did not substantially inhibit TSM contractions induced by carbachol, histamine, neurokinin A, and 50 mM KCl. These findings indicate that: 1) NP-1815-PX inhibits guinea pig TSM contractions mediated through the TP receptor in addition to P2X4 receptor whose stimulation induces EP_1 receptor-related mechanisms, and 2) NP-1815-PX may be a useful therapeutic for asthma.

1-P-055

Eperisone treats pulmonary fibrosis via preferential cell death induction in lung fibroblasts

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Although the exact pathogenesis of idiopathic pulmonary fibrosis (IPF) is still unknown, the transdifferentiation of fibroblasts into myofibroblasts and the production of extracellular matrix components such as collagen, triggered by alveolar epithelial cell injury, are important mechanisms of IPF development. In the lungs of IPF patients, apoptosis is less likely to be induced in fibroblasts than in alveolar epithelial cells, and this process is involved in the pathogenesis of IPF. We used a library containing approved drugs to screen for drugs that preferentially reduce cell viability in LL29 cells (lung fibroblasts from an IPF patient) compared with A549 cells (human alveolar epithelial cell line). After screening, we selected eperisone, a central muscle relaxant used in clinical practice. Eperisone showed little toxicity in A549 cells and preferentially reduced the percentage of viable LL29 cells, while pirfenidone and nintedanib did not have this effect. In an *in vivo* study, eperisone inhibited bleomycin (BLM)-induced pulmonary fibrosis, respiratory dysfunction, and fibroblast activation. In contrast, pirfenidone and nintedanib were less effective than eperisone in inhibiting BLM-induced pulmonary fibrosis under this experimental condition. Finally, we showed that eperisone did not induce adverse effects in the liver and gastrointestinal tract in the BLM-induced pulmonary fibrosis model. Considering these results, we propose that eperisone may be safer and more therapeutically beneficial for IPF patients than current therapies.

1-P-056

Effects of dexamethasone and roflumilast on neutrophilic asthma induced by antigen and cigarette smoke in mice

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Cigarette smoking in asthmatics is known to cause severe symptoms, pulmonary dysfunction, neutrophilic inflammation, and decreased glucocorticoid sensitivity. The differential effects of dexamethasone (Dex) and roflumilast (Rof) on airway inflammation, lung function and airway responsiveness in ovalbumin-induced asthmatic mice with or without concurrent cigarette smoke (CS) exposure were examined.

In the CS-exposed asthmatic mice, the airway neutrophils and lung compliance were increased, and the central airways resistance (Rn) and airway hyperreactivity (AHR) were decreased when compared with the asthmatic mice. Dex inhibited the airway eosinophils, airway resistance (Raw) and AHR in the asthmatic mice. In the CS-exposed asthmatic mice, Dex reduced the airway eosinophils but exacerbated the airway neutrophils, lung tissue resistance and AHR. Rof decreased the Rn in the asthmatic mice, but did not affect in the CS-exposed asthmatic mice.

Based on these results, in asthmatic mice with CS exposure, Dex was effective in reducing eosinophilic inflammation but exacerbated the neutrophilic inflammation, lung tissue resistance and AHR. Rof was ineffective in improving inflammation and lung function in asthmatic mice with CS exposure.

1-P-057

Role of histone ubiquitination in virus infection

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Virus infection may affect the epigenetic regulations in host cells, including post-translational histone modifications. Ubiquitination of histone H2B, has been reported to be involved in transcription activation. However, it remains unknown the role of histone ubiquitination in the pathology of virus infection. CNOT4 that is a component of the CCR4-NOT complex has a ubiquitin transferase activity at the RING domain (L16). Here, we found that CNOT4 was responsible for histone H2B ubiquitination in the host cells, which was linked to H3K4 methylation. In addition, upon influenza virus infection CNOT4 interacted to virus protein, resulting in the loss of H2B ubiquitination and H3K4 methylation. Moreover, the cells with a mutation in L16 of CNOT4, have increased virus replication. These results suggest that the CNOT4 is involved in the virus replication through histone H2B ubiquitination.

1-P-058

Development of artificial antibody against receptor binding domain of SARS-CoV-2 spike protein.

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Affibody is a class of smallest antibodies consisted of 58 amino acids, of which 13 amino acids are variable. Phage display is currently used for the production of affibody, but this method needs huge library construction for each antigen. In this study, we tried to develop the affibody against receptor binding domain (RBD) of SARS-CoV-2 spike protein *in silico*. SARS-CoV-2 virus binds to human ACE2 via spike protein, which is, therefore, a promising target for preventing SARS-CoV-2 infection. We analyzed three-dimensional coordinates of RBD structure and set antibody binding site on the interface surface between RBD and ACE2. We then designed many affibody structures based on the electrostatic potential of the binding site on RBD, and performed *in silico* docking prediction of constructed affibodies and RBD. Thereafter, two affibody molecules that showed the highest and second highest docking score as well as two molecules with much lower scores were expressed by wheat germ cell-free protein synthesis method. Binding affinity between expressed affibody molecules and RBD was examined with AlphaScreen assay, and two high score affibody molecules showed significantly higher luminescent signals due to binding than control low score molecules. In contrast, all four molecules showed no binding signals to negative control DHFR. The present study shows the successful non-animal-derived, cell-free and *in silico* generation of artificial antibody, targeting precise protein surface of SARS-CoV-2 RBD.

1-P-059

Rat basophilic leukemia cell-derived chymase interacts with SRAS-CoV-2 spike protein

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Mast cells are important sentinel cells in the first line of host defense against viral and bacterial entry. SARS-CoV-2 can activate mast cells present in the respiratory tract in the initial stage of the disease. In the present study, we attempted to dissect the mechanisms underlying the interaction of the function of mast cells, especially focusing on the mast cell-derived chymase, with the cell entry of SRAS-CoV-2 by using a well-established mast cell line, rat basophil leukemia (RBL-2H3) cells. RBL-2H3 cells were infected by pseudovirons and the viral entry was evaluated. The interactions between SRAS-CoV-2 spike and mast cell-derived chymase were observed and predicated binding-site were determined by using spike-truncating variants. During the viral infection, the interaction of chymase with SRAS-CoV-2 may have both detrimental and positive impacts. Whether SRAS-CoV-2 spike is a potential substrate of chymase, and if its cleavage modulates biological properties of spike-bearing virus would be focused on in future planned study.

1-P-060

Involvement of hematopoietic PGD_2 synthase for delayed wound healing in Streptozotocin-induced diabetic mice

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Delayed wound healing is a major problem in patients with diabetes melitus, which significantly impairs their quality of life. Prostaglandin (PG) D_2 is a major inflammatory lipid mediator synthesized by hematopoietic PGD_2 synthase (HPGDS) from PGH_2, a common precursor of all of PGs. In the present study, we investigated the role of PGD_2 in cutaneous wound healing in streptozotocin (STZ)-induced diabetic mice. C57BL/6 mice were injected with 50 mg/kg of STZ intraperitoneally daily for 5 days. Four weeks after the injection of STZ, a full thickness wound was created with an 8-mm diameter biopsy punch on the dorsal of mice showing the hyperglycemia (>300 mg/dL). Wound healing was significantly decelerated in diabetic mice compared with non-diabetic mice. The mRNAs of HPGDS, Cyclooxygenase (COX) 1, COX2, DP1 and DP2 receptors in mouse skin were measured by quantitative PCR. The skin of diabetic mice had significantly increased mRNAs of HPGDS and DP2 receptors as compared with the skin of non-diabetic mice. In addition, there was no significant change in the amount of DP1 receptors mRNA and COX1 mRNA, but the amount of COX2 mRNA tended to increase. In addition, immunohistochemical analysis revealed that HPGDS was upregulated in epidermal Langerhans cells of diabetic mice. These results suggest that in hyperglycemic skin, production of PGD_2 is increased in Langerhans cell and may be involved in delayed inflammation via DP2 receptors.

1-P-061

Topical application of celecoxib inhibits skin fibrosis and the expression of α- smooth muscle actin in bleomycin-induced sclerodema model mice

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Systemic sclerosis (SSc) is a connective tissue disorder characterized by the development of skin fibrosis. No topical treatment for the skin manifestations of SSc has been developed. We previously reported that celecoxib, a selective inhibitor of COX-2, and/or its derivative DM-celecoxib suppressed cardiac and renal fibrosis. Therefore, the effect of celecoxib on the SSc fibrosis was investigated. In *in-vivo* study, bleomycin was injected s.c. into a single location of the hair less mouse Hos:HR1 every 3 days for 10 days to induce scleroderma. For the treatment of celecoxib, celecoxib was dissolved into acetone and topically applied, whereas control mice received acetone only. Ten days after the first injection, skin section was obtained and histological analysis were performed. To clarify the mechanism of celecoxib action, normal human skin fibroblast cell line NB1RGB was stimulated with TGF-β to induced fibroblast-myofibroblast transformation and the effects of celecoxib on the expression of α-SMA was analyzed. We found that topical application of celecoxib significantly decreased skin thickness and the expression of α-SMA in bleomycin-induced scleroderma model mice. Consistent with these results, celecoxib inhibited the expression of α-SMA induced by TGF-β in NB1RGB cells. Further studies are now conducting to clarify the action of celecoxib.

1-P-062

Hochuekkito ameliorates LPS-induced anxiety-like behavior by suppressing interleukin-6 release

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Hochuekkito (HET), a Kampo medicine, is used to treat post-operative and post-illness general malaise and decreased motivation. HET is known to regulate immunity and inflammation. However, the mechanism underlying the anti-anxiety effect of HET is unknown. In this study, we revealed the effect of HET on lipopolysaccharide (LPS) - induced anxiety-like behavior and examined the mechanism underlying LPS induced anxiety-like behaviors. Following the administration of LPS (300μg/kg, i.p.), mice demonstrated inflammation-induced anxiety-like behaviors in hole-board and light-dark box tests. Systemic administration of LPS increased the serum levels of interleukin-6. Repeated administration of HET (1.0 g/kg, p.o.) once a day for 2 weeks before LPS treatment ameliorated anxiety-like behavior and reduced LPS-induced serum levels of interleukin-6 in 5 h after LPS treatment. Additionally, HET decreased LPS-induced secretion of interleukin-6 in human macrophage cell line (THP-1) and mouse macrophage cell line (RAW264.7) as in mouse models. Therefore, our findings suggest that HET may be useful in treating inflammation-induced anxiety-like behavior.

1-P-063

Effects of *Perilla* seed oil in combination with the nobiletin-rich ponkan powder on the cognitive function in healthy Japanese elderly: Possible supplementation for brain health in the elderly.

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We recently reported the potential impacts of *perilla* seed oil (PO), a rich source of a-linolenic acid (ALA, C18:3, n -3), on cognitive function in healthy elderly Japanese individuals. Here, supplements containing either PO alone or PO with nobiletin-rich air-dried immature ponkan powder were examined for their effects on brain functions in 49 healthy elderly Japanese individuals. PO group or the PO + ponkan powder (POPP) group for a 12-month randomized, double-blind, parallel-armed study. Taking a total of fifteen capsules daily for twelve months was not associated with any clinically significant side effects. From baseline to 12-months intervention, taking POPP significantly increased the cognitive index scores obtained by the three evaluation methods more than taking PO alone supplementation. The increased cognitive function observed in the POPP group was accompanied by increases in the levels of a-linolenic acid and docosahexaenoic acid in the erythrocyte plasma membranes and serum levels of brain-derived neurotropic factor (BDNF) and biological antioxidant potential. Our results demonstrate that a 12-months intervention with PO plus nobiletin-rich ADPP supplementations enhances serum BDNF and the antioxidant potential and may improve the age-related cognitive decline in healthy elderly individuals by enhancing the erythrocyte w-3 fatty acid levels.

1-P-064

Effects of *Anredera cordifolia* extract intake on learning and memory in senescence-accelerated mouse-prone 8 (SAMP8) mice

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Anredera cordifolia (AC) is a perennial plant of the Basellaceae family, and is attracting attention as a health food because it is easy to cultivate and has high nutritional value. So far, it has been reported that AC has an improving effect on lipid abnormalities and hypertension in mice and rats. However, it is not known how long-term administration of AC extract affects the central nervous function. In this study, we investigated the effects of an oral intake of AC extract on learning and memory using the senescence accelerated mouse-prone 8 (SAMP8) mouse.
SAMP8 mice (15 weeks old) were divided into two groups; a tap water intake group (CN: n = 10) and an AC extract water intake group (AC: n = 9) for 34 weeks under the free-feeding condition. Learning and memory function was assessed using Novel object recognition (NOR) task (age of 23 weeks) and the Morris water maze (MWM) task (aged 30 weeks). Following the completion of behavioral tests, blood biochemistry parameters and hippocampal levels of brain-derived neurotropic factor, postsynaptic density protein 95, NR2A, and p-cAMP-response element binding (CREB)/CREB ratio were measured. The AC group spent more time exploring the novel object in the NOR task, and showed better acquisition and retention in the MWM task than the CN group. In addition, AC elevated the levels of the aforementioned neuronal plasticity-related proteins, and did not affect blood biochemistry parameters. These results suggest that the AC extract may improve learning and memory in SAMP8 mice without causing any noticeable side effects on the body.

1-P-065

The preventive effect of *Actinidia arguta* (Sarunashi) juice on Parkinson's disease onset through antioxidative activity

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Parkinson's disease (PD) is a progressive neurodegenerative disease that exhibits motor dysfunction due to deficit of dopaminergic neurons in the substantia nigra. Oxidative stress is a factor involved in the onset and progression of PD. We have previously shown that *Actinidia arguta* has an antioxidative activity. In this study, we evaluated the effect of prophylactic administration of *Actinidia arguta* (Sarunashi) in MPTP induced PD mice model. Male C57BL/6J mice were allowed to continuously ingest water or Sarunashi juice from 7 weeks of age. At 8 weeks of age, MPTP (30 mg/kg/day) was intraperitoneally administered for 5 consecutive days. In the MPTP + water group, significant motor dysfunction was observed in the pole test, catalepsy test, beam walk test, and rotor rod test compared with the negative control (NC) group. However, in the MPTP + Sarunashi group, no impaired motor function was observed as compared with the NC group. The protein expression level of tyrosine hydroxylase (TH) in the MPTP + water group was lower than that in the NC group. On the other hand, TH expression in the MPTP + Sarunashi group was higher than that in the MPTP + water group. In conclusion, prophylactic administration of Sarunashi juice suppressed MPTP induced motor dysfunction mediated via decreased TH expression.

1-P-066

Atractylodin possesses anti-nociceptive effect through a long-lasting activation of TRPA1

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Atractylodin (ATR) is a bioactive component found in dried rhizomes of Atractylodes lancea (AL) De Candolle. Although AL has accumulated empirical evidence for the treatment of pain, the molecular mechanism underlying the anti-pain effect of ATR remains unclear. In this study, we found that ATR increased single channel activities of transient receptor potential ankyrin-1 (TRPA1) in hTRPA1 expressing HEK293 cells. A bath application of ATR produced a long-lasting calcium response, and which was completely diminished in the dorsal root ganglion neurons of TRPA1 knockout mice. Intraplantar injection of ATR evoked moderate and prolonged nociceptive behavior compared with allyl isothiocyanate (AITC). Systemic application of ATR inhibited AITC-induced nociceptive responses in a dose-dependent manner. Co-application of ATR and QX-314 increased the noxious heat threshold compared with AITC in vivo. Collectively, we concluded that ATR is a unique agonist of TRPA1, which produces long-lasting channel activation. Our results indicated ATR-mediated anti-nociceptive effect through desensitization of TRPA1-expressing nociceptors.

1-P-067

1-P-068

Effect of the liquid form of traditional Chinese medicine, Hozen-S, on gastric motility in dog

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Juzen-taiho-to, a traditional Chinese herbal medicine, is used for patients with anorexia and fatigue in human medicine. In our previous study, a granulated form of Juzen-taiho-to improved vincristine-induced gastrointestinal adverse effects through increasing gastric motility in dogs. However, the effect of Hozen-S, the sweet liquid form of Juzen-taiho-to, on dog gastric motility has not been investigated. Therefore, we examined whether the administration of Hozen-S to dogs affects their gastric motility. To further elucidate the mechanism of the effect of Hozen-S on gastric contraction, we assessed the plasma ghrelin level of the dog. Finally, we assessed the Hozen-S palatability compared to granulated Juzen-taiho-to in dogs and the effect on body weight in dogs. Administration of Hozen-S significantly increased gastric motility, plasma ghrelin concentration, and body weight in the dogs. As a result of the palatability evaluation, these dogs preferred Hozen-S to the granulated Juzen-taiho-to. In conclusion, Hozen-S administration to dogs promoted gastric motility by raising the plasma ghrelin level. Considering these functional and palatability data, Hozen-S might replace the granulated type Juzen-taiho-to and become a prominent traditional Chinese veterinary medicament.

1-P-069

G protein-coupled bile acid receptor TGR5 signaling regulates fibroblast growth factor 21

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Fibroblast growth factor 21 (FGF21), which structurally belongs to the FGF superfamily, acts as an endocrine factor and plays important roles in the regulation of energy homeostasis, glucose and lipid metabolism. The therapeutic administration of FGF21 analogs improves nonalcoholic steatohepatitis, the severe stage of non-alcoholic fatty liver disease, which is associated with obesity, metabolic syndrome, dyslipidemia and type 2 diabetes. In the present study, we screened crude extracts from 88 herbal drugs frequently used in Kampo prescriptions as potential anti-obesity agents by monitoring the production of FGF21 in C2C12 myotubes. Among these extracts screened, we found that 3-*O*-acetyloleanolic acid, an active constituent isolated from the fruits of *Forsythia suspensa*, stimulated FGF21 production in C2C12 myotubes. Additionally, significant increases in CRE-dependent luciferase activity were observed in cells overexpressing bile acid receptor TGR5 in response to 3-*O*-acetyloleanolic acid treatment, which indicated that the responses caused by 3-*O*-acetyloleanolic acid was dependent on TGR5 activation. We observed that the phosphorylation of p38 was increased by 3-*O*-acetyloleanolic acid rapidly in C2C12 myotubes. Pretreatment with the selective p38 inhibitor SB203580 also significantly repressed the stimulatory effect of 3-*O*-acetyloleanolic acid on FGF21 secretion. These findings collectively indicated that TGR5 receptor signaling up-regulates FGF21 expression via p38 activation.

1-P-070

Dihydromyricetin suppresses cell proliferation via ERK1/2 in human hepatocellular carcinoma cells.

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Purpose: Dihydromyricetin (DHM) is a flavonoid isolated from Hovenia dulcis. DHM have been reported to have hepatoprotective and anti-tumor effects. The purpose of this study was to elucidate the anti-tumor effect and the mechanism of DHM in human hepatocellular carcinoma cell line, Huh-7.

Methods: Huh-7 cells were cultivated with DHM (0 - 100 μM) for 72 hours. We evaluated that the cell proliferation in Huh-7 cells treated with DHM using a MTS assay. The mRNA and protein levels of Ki-67, cyclinD1, and caspase 3 were analyzed using a real time-RT-PCR or a western blot analysis. The protein levels and phosphorylation of ERK, c-fos, c-jun as MAPK signaling were confirmed.

Results and discussion: Although DHM (1 - 10 μM) did not changed cell proliferation, 30 μM and 100 μM of DHM significantly decreased in Huh-7 cells.

Ki-67 and cyclin D1 mRNA levels were decreased in DHM-treated Huh-7 cells. However, caspase 3 mRNA levels was not increased. Cyclin D1 protein levels were also significantly decreased and caspase 3 protein levels were not increased in DHM-treated with Huh-7 cells; these suggest that DHM inhibited cell proliferation and did not effect the apoptotic protein, caspase 3.

The total ERK and pERK were significantly decreased in Huh-7 treated with DHM. However, the level of p-c-fos, and p-c-jun were tended to decrease. DHM may related to the decreases of HCC cell proliferation through the ERK.

1-P-071

Development of a method for evaluating pain in sensory neurons using 236,880 electrode CMOS-MEA

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Toxicity of the compound on the nervous system has been reported in development of pharmaceuticals such as toxicity of the compound and medicinal efficacy evaluation. In nonclinical study for the purpose of pharmaceuticals development, in in vivo using model animals are being conducted to examine the efficacy of the drug. However, the labor required for the experiment is excessive and it has a challenges low reproducibility and accuracy. Therefore, there is a need to develop a high accuracy and objective method of pain evaluated, but there method are not established. In this study, we aimed to development of pain evaluation system to evaluate of effectiveness and side effect of drugs. Using complementary metal-oxide semiconductor microelectrode array (CMOS-MEA) with nerve cells electrical activity can measure an ultra-high temporal resolution, the response of single sensory neurons to pain was obtained. Furthermore, based on the electrical activity obtained from 236,880 electrodes, we constructed a method to identify single cell, calculation of conduction velocity of axons and profiling of firing pattern each single cells.

1-P-072

Ready-to-use human neuron plates for pharmacological assays～ 384 well 2D culture plates～

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In vitro pharmacological assay based on human iPSC-derived neurons is a valuable alternative to the standard animal models. One of the main methods for this purpose is imaging drug-induced Ca^{2+} responses in neuronal networks cultured on multi-well plates. Preparing iPSC-derived neurons, however, requires costly optimization and lengthy culturing, and one possible solution to this problem would be to fabricate neuron plates that can be transported from the manufacturer to the user.

We have developed transportable neuron plates for high-throughput screening by optimizing the plate coating and culture conditions. Ca^{2+} imaging showed that human iPSC-derived neurons cultured in these plates exhibit excellent drug responses.

The transportable plates provide ready-to-use human neuron assay systems and would contribute to accelerate drug development.

1-P-073

seizure liability prediction method based on electrical activities in human iPS cell-derived neurons using machine learning -Comparison of raster plot model and parameter model-

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In vitro microelectrode array (MEA) assessment using human induced pluripotent stem cell (iPSC)-derived neurons holds promise as a method of seizure and toxicity evaluation. However it is difficult to detect the response of drugs with different mechanisms of action with a single parameter, and the analysis method has become an issue. Therefore, in this study, we developed an artificial intelligence (AI) model that learned raster plot images of electrical activity acquired by multiple electrodes and an SVM model that learned parameters calculated from time-series data of electrical activity. We compared the accuracy of predicting convulsive toxicity for each of the developed models. To train the model, extracellular potential data of co-cultured samples of human iPSC-derived cortical neurons and stellate cells obtained using MED64 Presto were used. In the SVM model using the spike time-series information and burst-related parameters, the risk assessment of the positive and negative compounds of the trained data was achieved, but the untrained data of acetaminophen was judged to be positive. In contrast, AI accurately predicted seizure risk, even with unlearned well data. These results indicated that using raster plot features method is useful for predicting the seizure liability using hiPSC-derived neurons.

1-P-074

Toxicity risk assessment of pesticide-related compounds using human iPSC-derived neurons

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In vitro human iPSC-derived neuronal systems are an alternative platform for neurotoxicity testing to animal models and primary cultures. Microelectrode array (MEA), measurement system of the electrophysiological activity, are suitable to evaluate the neurotoxicity of compounds. We have previously reported the electrophysiological responses to known neurotoxicity compounds using MEA in human iPSC-derived neurons. However, the identification of analytical parameters to detecting toxicity of unknown compounds remains an important issue. We identify the analytical parameters enabling the separation of responses between neurotoxicity and negative control, and the separation the mechanism of action by using principal component analysis. By comparing the optimized analytical parameters of each testing compound with the standard deviation (SD) of negative control, we can predict general neurotoxicity in relatively quantitatively scale, for example, low risk for lower than SD range, medium risk for 2xSD, and high risk for over 2xSD. This predictability can help select appropriate concentration levels to avoid toxicity/adverse effects. In this study, we evaluated the toxicity risk of pesticides and industrial chemicals. We demonstrated that the toxicity risk can be detected by MEA measurement of human iPSC-derived neurons.

1-P-075

Comparison of the Benchmark Dose (BMD) approach by four different type software with No Observed Adverse Effect Level (NOAEL) approach for tumorigenicity in rodent bioassays of pesticides in Japan

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Benchmark Dose (BMD) approach is the way to calculate the risk at the lower dosage of chemical exposure, applying a mathematical model to the dose-response relationship. Although a number of the guidelines and software have been developed worldwide, the harmonization of BMD application is still undergoing. Before applying in actual risk assessment, it is essential to evaluate whether the BMD and NOAEL approaches give the same range of POD values and how different BMD values the major BMD software would give.

Here, we calculated the lower limit of BMD confidence interval (BMDL) from 201 tumorigenicity data publicized in the pesticide risk assessment reports by the Food Safety Commission of Japan (FSCJ). We applied three well-known BMD software, PROAST, BMDS, and BBMD, to compare their BMDLs to NOAELs and LOAELs and between the recently implemented methodologies such as model averaging (MA) or Bayesian inference.

Our result indicates that the BMD approach gives Point of Departure (POD) similar to the NOAEL approach if the data applied show a clear dose-response relationship. However, most of the datasets that resulted in failed calculation or extremely low BMDLs showed unclear dose-response relationships, such as non-monotonous and sporadic responses. We also noted that the Bayesian inference software gave failed calculation or extreme BMDLs less than the frequentist approaches.

1-P-076

Molecular effects of cigarette smoke on airway epithelium cells

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Cigarette smoking is one of the risk factors in cardiovascular and respiratory diseases including atherosclerosis and chronic obstructive pulmonary disease (COPD). Although cigarette smoke mainstream consists of more than 4,500 chemical compounds, the compounds responsible for these diseases are still unknown. Cigarette smoke is divided in two phases: tar (particle) phase containing nicotine and gas phase. Airway epithelium cells are exposed both tar and gas phases. In this study, we have examined the effect of tar and gas phase of cigarette smoke on airway epithelium cells. The gas phase extract of cigarette smoke was prepared as previously described (Higashi et al., 2014, PLOS ONE 9: e107856). The tar phase extract of cigarette smoke was prepared by extracting tar phase on Cambridge filter with DMSO. Both tar and gas phases induced cell death in airway epithelial cells. The cell death induced by the gas phase was PKC-dependent, whereas the tar phase induced DNA double strand break and PKC-independent cell death. The pharmacological experiments revealed that the airway epithelium cell death by gas phase were ferroptosis. According to Yoshida et al., ferroptosis is involved in COPD pathogenesis (Yoshida et al., 2019, Nat Commun 10: 3145). Taken together, cigarette smoke gas phase might be a critical factor for cigarette smoking-induced COPD onset and development.

1-P-077

L-type amino acid transporter 1 inhibitor JPH203 as a new therapeutic target for castration resistant prostate cancer treatment

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LAT1 (SLC7A5) is a type of amino acid transporter that transports many essential amino acids, including Leucine, into cells. High expression of LAT1 is associated with poor prognosis in a variety of carcinomas, and a selective inhibitor of LAT1 (JPH203) has been reported to inhibit cancer cell growth. Recently, we reported that LAT1 expression is upregulated in the cell line C4-2, a model of castration-resistant prostate cancer (CRPC). We hypothesized that JPH203 could be a novel therapeutic agent for CRPC. We examined the effects of JPH203 in C4-2 and PC-3 cell lines as a model of CRPC and LNCaP cell line as hormone-sensitive prostate cancer (HSPC). We performed [14C] Leucine uptake assay for functional analysis and WST-8 assay for cytotoxicity test. In addition, Migration/Invasion assay was performed using Corning™ Falcon™ Cell Culture Inserts to evaluate cell migration and invasion ability. To elucidate the molecular mechanism of the inhibitory effect on proliferation, the presence or absence of mTOR pathway inhibition was verified using Western blotting. We also investigated the tumor suppressive effect of JPH203 in mice injected subcutaneously with C4-2 cells. As a result, Leucine uptake was predominantly decreased by JPH203 in C4-2 and PC-3 cells, and the inhibitory effect on cell proliferation was also confirmed. In C4 -2 cells, JPH203 inhibited the mTOR pathway. JPH203 suppressed tumor growth in vivo. These results suggest that JPH203 may have an antitumor effect on CRPC and may be a novel therapeutic agent.

1-P-078

Pro-apoptotic protein p75-NTR associated cell death executor (NADE) expression in chemical carcinogenesis of the rat liver

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Data for gene expression profile in the rat liver (after exposure to 150 chemical compounds) are available from Open TG-GATES database generated from the Toxicogenomics Project of Japan. With the 150 chemical compounds, several carcinogenic agents induced the expression of p75-NTR associated cell death executor (NADE) in the liver. NADE is known as a pro-apoptotic protein and induces apoptosis by associating with a partner protein, including p75-NTR, hamartin, or Smac, under certain physiological conditions. However, none of these genes were induced in these cases. As NADE expression starts to increase as early as three days of exposure to the carcinogenic agents, NADE is a potential early marker for chemical carcinogenesis. To evaluate whether NADE can be a carcinogenic marker, we examined the role of NADE in the process of chemical carcinogenesis. In our study, Sprague-Dawley rats were treated with a genotoxic carcinogen, diethylnitrosamine, for up to 8 weeks followed by another 4 weeks of no treatment. The liver was excised to obtain RNA and protein samples and paraffin embedded tissue sections. The expression of NADE and some cancer markers was quantified by real time PCR. By immunohistochemistry and TUNEL assay, the expression pattern of NADE, some cancer markers was visualised and apoptosis was detected. The NADE expression correlated with development and growth of cancerous cells, but not with apoptosis. Here, we discuss the role of NADE expression in cancerous growth and apoptosis.

1-P-079

Development of liver metabolism and excretion test method utilizing a collagen vitrigel membrane: Advantages of HepG2-NIAS cells with enhanced liver-specific function and structure by oxygenation culture

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We reported the enhanced liver-specific function and structure of HepG2 cells by oxygenation culture via a collagen vitrigel membrane (Oshikata-Miyazaki and Takezawa, 2016). The cells were conditioned in our laboratory for a long period, so their characteristics may change from the original HepG2 cells registered in RIKEN cell bank (RCB) with the number of 1648 (HepG2-RCB1648 cells). We named the conditioned HepG2-RCB1648 cells in our laboratory as HepG2-NIAS cells. The aim of the present study is to clarify the features of HepG2-NIAS cells by comparing to HepG2 cells with two different cell culture histories. HepG2-NIAS cells subjected to oxygenation culture grew as a monolayer and showed a high CYP3A4 activity which was equivalent to almost half level to that of differentiated HepaRG cells and potential to form bile canaliculus-like structure. On the other hand, HepG2-RCB1648 cells and HepG2-RCB1886 cells subjected to oxygenation culture formed large multicellular aggregates and almost no detectable CYP3A4 activity and potential to form bile canaliculus-like structure. Protein expression levels of sinusoidal drug uptake and canalicular transporters were notably higher in HepG2-NIAS cells than HepG2-RCB1648 cells and HepG2-RCB1886 cells subjected to oxygenation culture. In contrast, protein expression levels of sinusoidal drug efflux transporters were highest in HepG2-RCB1648 cells. In conclusion, it is expected that HepG2-NIAS cells subjected to oxygenation culture are useful for predicting drug metabolism and excretion in the liver.

1-P-080

Evaluation of the anti-glycation effect and skin protection effect by a supplement containing brown algae extract powder. (A randomized,Double-blind,Placebo-controlled, Parallel group study)

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Glycation stress is a concept of biological stress caused by excess sugars, aldehyde and its subsequent reactions in the body. Especially, it is thought that accumulation of Advanced Glycation End products (AGEs) cause skin yellowing, deep wrinkles and a decrease in skin elasticity due to crosslinking to collagen or elastin in the dermis.
We have reported that phlorotannins, polyphenols unique to brown algae, could inhibit the formation of N-ε-carboxymethyllysine resulting from glycation of collagen.
The aim of this study was to investigate the anti-glycation and skin protection effects against UV exposure by long-term intake of the brown algae extract powder containing phlorotannins as a supplement.
This study have performed a randomized, double-blind, placebo-controlled, parallel group study.
Forty-two healthy Japanese adult female were randomly divided into two groups: one group consuming supplements containing brown algae extract powder (Phlorotannins intake group) and the other group consuming supplements not containing brown algae extract powder (placebo group). In both groups, each supplement was consumed daily, and blood AGEs concentration and Minimal Erythema Dose were measured every 4 weeks. In addition, the condition of skin was analyzed by using the skin image analyzer.
In this presentation, we will introduce the skin protection effect against UV exposure and the anti-glycation effects by consuming the brown algae extract powder for eight weeks.

1-P-081

Association between effect of duloxetine on chronic orofacial pain and expression of platelet serotonin transporter in patients with burning mouth syndrome and atypical odontalgia

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Introduction: Burning mouth syndrome and atypical odontalgia (BMS/AO) are chronic orofacial pain conditions in the absence of any identifiable odontogenic pathology. The pain is treatment-resistant and frequently causes depressive symptoms. Duloxetine (DLX), a serotonin-noradrenaline reuptake inhibitor, is not only used as therapy for depression, but also for chronic orofacial pain. Since their mechanisms in detail remains unknown, in this study, we focused on serotonin transporter (SERT), one of DLX action site, and investigated association between expression of SERT and effect of DLX on pain in BMS/AO.

Methods: The patients with BMS/AO, were assessed for severity of pain using the visual analog scale (VAS) and for signs of depression using the Hamilton Depression Rating Scale (HDRS). In their platelets before (baseline) and 12 weeks after DLX-treatment, the expression of total and ubiquitinated SERT proteins was confirmed by Western blot. This study was approved by the Ethics Review Committees of Nagoya, Aichi Gakuin, and Meijo Universities.

Results: The expression of total and ubiquitinated SERT protein at baseline in all patients were higher and lower, respectively, compared to those in controls. After the DLX-treatment, there was no difference in the total SERT protein levels between the patients and controls. The mean of VAS and HDRS scores or the expression of total SERT protein were significantly decreased after the treatment, compared to those at baseline.

Conclusions: These results indicate that DLX relieves chronic orofacial pain in patients with BMS/AO, and such effect may be mediated via SERT downregulation.

1-P-082

Effects of a novel hepatitis B antiviral drug in renal organic acid transporters

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In treatment of hepatitis B virus (HBV), it is usually difficult for us to control with emergence of drug resistance. As HBV often reactive after treatment was stopped, patients must keep it for long term. Recently, we have developed E-CFCP, as a candidate drug of HBV for patients with drug-resistant HBV. As it has high antiviral activity and the half-life also is longer, patients can take it in a once-weekly dosing. We expect that E-CFCP can greatly improve the quality of life of patients. However, effects of E-CFCP are unclear in renal. The aim of this study is to clarify the effects of E-CFCP in the kidney, especially organic acid transporter (Organic anion transporters：OATs, Organic cation transporter：OCT) . We conducted cell viability studies using mouse-derived renal cortical cells (S2, CCD, cTAL) and uptake studies using radioisotopes to determine the effects of E-CFCP on the kidneys. In cell viability studies, E-CFCP has no cytotoxicity in all cell lines. We also examined the effect of drugs at high concentration using S2 cells. E-CFCP has no cytotoxicity even at high concentrations. In the substrate uptake assay, there was no inhibition of substrate uptake by E-CFCP, the transporter is not involved in the intracellular transport of E-CFCP and is unlikely to cause cytotoxicity. In conclusion, E-CFCP, a novel HBV antiviral drug, is unlikely to cause renal damage. It may be a novel great candidate drug of HBV for patients with drug-resistant HBV.

1-P-083

Possible involvement of transient receptor potential melastatin 4 channels in the adrenergic contraction in mouse prostate smooth muscles

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It has been suggested that transient receptor potential melastatin 4 (TRPM4) cation channels are abundantly expressed in the prostate gland. However, the precise role of TRPM4 channels in prostatic smooth muscle contractility is still unknown. Here, we examined if TRPM4 channels are involved in the adrenergic contraction in mouse prostatic smooth muscles. Adrenergic contractile responses evoked by electrical field stimulation of intrinsic sympathetic nerve or exogenously applied noradrenaline (NA) were isometrically recorded and effects of 9-phenanthrol, a specific and potent TRPM4 channel inhibitor, on those contractile responses were investigated in mouse ventral prostate preparations. 9-phenanthrol (10 and 30 μM) concentration-dependently inhibited both sympathetic nerve-evoked contractions and NA-induced contractions. The percentage inhibition by 9-phenanthrol was much greater at lower stimulus frequencies and lower NA concentrations. However, the agent did not inhibit NA-induced contractile response due to release of stored Ca^{2+}. These results suggest that TRPM4 channels are involved in the adrenergic contraction possibly through membrane depolarization by their opening and seems to be a potential candidate for the treatment of benign prostatic hyperplasia.

1-P-084

Role of TRPV1 in hyaluronan-induced apoptosis avoidance in MCF-7 cells

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Hyaluronic acid (HA) is produced at extremely high levels in some breast cancer cells, which can then escape from apoptosis even if they become detached from the extracellular matrix. Activation of TRPV1, a non-specific cation channel protein, has been shown to cause apoptosis in these cells. Therefore, using MCF-7 cells, we investigated the expression of the HA receptor CD44 and TRPV1 when the cells were placed on a low-adhesive scaffold. Then, we examined the effects of the HA-synthesis inhibitor 4-methylumbelliferone (4-MU) on TRPV1 expression as well as the effect of TRPV1 modulators on 4-MU–induced apoptosis. We also tested the direct action of HA on TRPV1 activity by using a Ca imaging system. In cancer spheroids formed on the poly (2-hydroxyethylmethacrylate)-coated dishes, both HA production and CD44 mRNA expression were increased, whereas TRPV1 mRNA expression was decreased. 4-MU inhibited both HA production and CD44 expression but increased TRPV1 mRNA expression and protein production. The TRPV1 agonist capsaicin increased the amount of Annexin V/PI-positive cells, the action of which was inhibited by the TRPV1 antagonist AMG9810. Furthermore, 4-MU–induced apoptosis was strongly suppressed by AMG9810. HA itself inhibited the capsaicin-induced increase in Ca^{2+} in TRPV1-transfected HEK293 cells. These results suggest that during cell detachment, the HA-CD44 pathway is likely to be involved in the underlying mechanism of apoptosis avoidance by inhibiting the expression and function of TRPV1.

1-P-085

Phosphorylation of Annexin A2 is involved in activation of AKT upon endothelin-1 stimulation in melanoma cells

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Overexpression of endothelin (ET)-1 and endothelin receptors (ETRs) are associated with human cancer malignancy. In cancer cells, ET-1 activates various signaling pathways, including mitogen-activated protein kinase pathway, phosphatidylinositol 3-kinase pathway, and protein kinase C pathway, through ETRs, although the mechanisms by which ET-1 activates these signaling pathways remain unclear. We previously reported that ETRs interacted with annexin A2, which is overexpressed in various cancers, and *annexin A2* silencing suppressed ERK activation upon ET-1 stimulation in human umbilical vein cell line, EA.hy926 cells. Here we examined roles of annexin A2 in ET-1 signaling pathway of melanoma cells. In melanoma cells, ET-1 stimulation activated ATK, and phosphorylated serin residues of annexin A2. *Annexin A2* silencing suppressed activation of AKT upon ET-1 stimulation. In addition, we found that serine residues mutant of annexin A2 suppressed activation of AKT. Our results suggested that phosphorylation of annexin A2 plays important roles in AKT activation of melanoma cells upon ET-1 stimulation.

1-P-086

Dephosphorylation of $GABA_B$ receptor subunit by over-expression of protein phosphatase 5.

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[Purpose] GABAB receptors are responsible for inhibitory transmission by conjugating with Gi proteins. In addition, this receptor functions by forming a dimer of R1 and R2 subunits (R2). Phosphorylation of serine residue at position 892 (S892) of R2 suppresses receptor internalization and desensitization. We have investigated the possibility that protein phosphatase 5 (PP5) dephosphorylates S892. In this study, we investigated the effect of overexpression of PP5 on the phosphorylation level of S892.
[Methods] pCDH-CMV-MCS-EF1copGFP-PP5 was prepared, packaged in lentivirus, and infected with HEK293 cells. The PP5 and R2 genes were simultaneously introduced into HEK293 cells, and the phosphorylation level of S892 was confirmed by Western blotting.
[Results] Virus packaging of the R2 gene was confirmed, but sufficient infection was not obtained. On the other hand, when PP5 and R2 were transiently co-expressed in HEK293 cells, a decrease in the phosphorylation level of S892 was confirmed in the PP5 overexpression group.
[Conclusion] If the detailed function of internalization or inactivation mediated by GABAB receptor dephosphorylation due to overexpression of PP5 is clarified, multiple sclerosis and amyotrophic lateral sclerosis associated with GABAB receptors will be clarified. It may lead to detailed elucidation of pathological conditions such as sclerosis and development of new therapeutic agents.

1-P-087

Dual regulation of ERK phosphorylation by activation of G_q-protein- and arrestin-mediated pathways via histamine H_1 receptors

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G_q-protein-coupled histamine H_1 receptors play a key role for allergic and inflammatory reactions. We constructed two differential C-terminal mutants of human H_1 receptors, S487TR and S487A, in which the Ser487 residue of C-terminal was truncated or mutated to alanine, respectively. We have found that S487TR and S487A selectively couples to G_q-protein and β-arrestin, respectively. In this study, we investigated histamine-induced and G_q-protein/β-arrestin-mediated ERK phosphorylation in Chinese hamster ovary cells expressing S487TR and S487A. In cells expressing S487TR, histamine transiently induced ERK phosphorylation, which were suppressed by intracellular Ca^{2+} chelator and inhibitors of protein kinase C (PKC) but not inhibitors of G-protein-coupled receptor kinase (GRK), clathrin, Raf and MEK. In contrast, histamine sustainably induced ERK phosphorylation in cells expressing S487A, which were suppressed by inhibitors of GRK, clathrin, Raf and MEK but not intracellular Ca^{2+} chelator and PKC inhibitors. These results suggest that progressive processes of the H_1-receptor-mediated ERK phosphorylation might be differentially regulated by the G_q-protein/Ca^{2+}/PKC- and GRK/β-arrestin/clathrin/Raf/MEK-dependent pathways.

1-P-088

Contributions of two enantiomers of the citalopram-induced blockade of Nav1.5 voltage-gated sodium channels current

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Citalopram has been reported to have cardiac adverse effects. Although citalopram is known to be a racemic compound comprised of S-citalopram (escitalopram) and R-citalopram, it is still unclear which enantiomer is responsible for cardiac adverse effects induced by citalopram. In the present study, we investigated whether citalopram, escitalopram and R-citalopram had an electrophysiological effect on Nav1.5 voltage-gated sodium channel (VGSC) current and how their electrophysiological properties affected Nav1.5 VGSC. When whole-cell patch clamp was performed for analysis, the IC_{50} of citalopram, escitalopram and R-citalopram were 89.2, 58.6 and 174.0 μM, respectively. In addition, treatment with 100 μM citalopram and escitalopram changed the voltage-dependence of activation and induced a negative shift of the voltage of half-maximal activation compared to 100 μM R-citalopram. In contrast, treatment with 100 μM citalopram and escitalopram changed the voltage-dependence of inactivation, and the voltage at half-maximal inactivation slightly shifted toward negative potential. These results suggest that the adverse cardiac effect produced be citalopram might result from modification of the electrophysiological properties of Nav1.5 VGSCs, and escitalopram might contribute more to this adverse effect than R-citalopram.

1-P-089

Reactivity of TRPA1 to menthol differs between mouse and dog

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TRPA1 is a non-selective cation channel and has been shown to be activated by a wide variety of noxious compounds and physiological stressors. Interestingly, TRPA1 is reported to be stimulated by menthol, an agonist of TRPM8, with different dose dependencies between mouse and human. It has been suggested that this different reactivity may be attributable to three amino acid residues in the TM5 region of TRPA1, namely, S–T–V in human and S–T–G in mouse. In this study, to further investigate the importance of the TM5 region through comparison with other mammalian species, canine TRPA1 cDNA was cloned and its reactivity to several agonists was compared in dog and mouse by using recombinant proteins. The TM5 region of cloned canine TRPA1 showed high similarity to that of human TRPA1 and the S–T–V residues were conserved. HEK293T cells were transfected with mouse or canine TRPA1 and subjected to calcium influx imaging. Both mouse and canine TRPA1 were activated by the TRPA1 agonist allyl isothiocyanate and were inactivated by the TRPA1 antagonist HC-030031. In contrast, reactivity to menthol was observed to differ between these two species. Mouse TRPA1 was activated by 100 μM of menthol and showed transient Ca^{2+} influx when menthol was washed out ("off response"), whereas canine TRPA1 activation required a high (300 μM) concentration of menthol, but no off response was observed. These results showed that the reactivity of canine TRPA1 against menthol is similar to that of human TRPA1 but not to mouse TRPA1, which might reflect the similarity of their respective TM5 regions.

1-P-090

The role of TRPA1 in hypoxic response of the carotid body

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The carotid body is a peripheral chemoreceptor located in the branches of the internal and external carotid arteries, and has long been known to be involved in oxygen sensing. However, the precise molecular mechanism of oxygen sensing remains unclear. Our group has previously identified that TRPA1, a type of non selective cation channel, is activated in hypoxic conditions. To understand whether TRPA1 contributes to oxygen sensing in the carotid body, we have performed immunohistochemistry experiments, which revealed that TRPA1 is expressed in the carotid body. Furthermore, Ca^{2+} imaging experiments suggested that TRPA1 in the carotid body did not contribute to the response to severe hypoxia, but the respond to moderate hypoxia. Taken together, our results suggest that TRPA1 may play a role in the oxygen sensing mechanism in the carotid body.

1-P-091

Voltage-gated chloride channels control mitochondrial membrane potential

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Voltage ($\Delta\Psi$) across the inner mitochondrial membrane (IMM) controls a variety of mitochondrial function including ATP synthesis, thermogenesis, and cell death. Thus, maintenance of $\Delta\Psi$ and stability of its magnitude are of a paramount significance for the physiology of the cell and the entire organism. Currently, the electron transport chain remains the only well-established mechanism for the $\Delta\Psi$ maintenance. Here, we identify mitochondrial Cl^- channels as a crucial mechanism for the $\Delta\Psi$ maintenance. We use the whole-IMM patch-clamp analysis, and demonstrate that mitochondria possess two distinct types of voltage-gated Cl^- channels (Cl_V), inactivating Cl_V activated at hyperpolarized voltage (hCl_V) and non-inactivating Cl_V activated at depolarized voltage (dCl_V). hCl_V is characterized by low activation threshold just below the physiological $\Delta\Psi$ values and has fast inactivation. hCl_V is a novel mitochondrial Cl^- channel that has never been reported previously. In contrast, dCl_V activated only by profound membrane depolarization to ~ 0 mV and is completely lacking inactivation. dCl_V likely corresponds to the inner membrane anion channel or the 108-pS anion channel, but its detailed electrophysiological analysis was missing. Using optical methods and mitochondrial respiration assays, we demonstrate that mitochondrial Cl^- channels largely ameliorate $\Delta\Psi$ depolarization induced by mitochondrial uncouplers (H^+ leak) and Ca^{2+} uptake via the mitochondrial Ca^{2+} uniporter. Importantly, mitochondrial Cl^- channels profoundly delay the activation of mitochondrial permeability transition pore. Thus, hCl_V and dCl_V represent a previously unknown mechanism for $\Delta\Psi$ maintenance, which could play a fundamental role in preserving mitochondrial integrity and function.

1-P-092

One amino acid mutation of prostanoid EP4 receptor altered its signaling profiles.

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The prostanoid EP4 receptor belongs to the family of G protein-coupled receptors and it has been reported that the signaling pathway mediated by the EP4 receptor contributes to the cancer development. The cytoplasmic region of the EP4 receptor has several characteristic sites that are significantly different from other prostanoid receptors. In this study, we focused on one amino acid and examined the effects of point mutation on EP4 receptor signaling pathway. Thus, the point mutation-introduced EP4 receptor-stably expressing HEK cell line was prepared, and comparative experiment were conducted with wild-type EP4 receptor-expressing cell line. As a result, constitutive cAMP production and ligand-independent cell proliferation retardation were observed in the mutant EP4 receptor-expressing cell line when compared with wild-type EP4 receptor expressing cell line. These results suggested that the introducing only one mutation of the specific amino acid of the EP4 receptor would have a possibility to suppress the EP4 receptor-initiated cancer development by delaying the growth of cells.

1-P-093

Metabolites of Prostaglandin D_2 act as biased agonists for D type prostanoid receptors

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The Gs protein coupled D type prostanoid (DP) receptors have been known to exhibit anti-inflammatory effects.
The DP receptors are well recognized as cognate receptors for prostaglandin (PG)D_2, which is known to be involved in inflammatory responses and allergies.
There are five endogeneous metabolites of PGD_2; PGJ_2, Δ^{12}-PGJ_2, 13,14-dihydro-15-keto-PGD_2, 15-deoxy-$\Delta^{12,14}$-PGD_2, and 15-deoxy-$\Delta^{12,14}$-PGJ_2.
Previously, we showed that 15-keto-PGE_2, a metabolite of PGE_2, acts on EP2 and EP4 receptors as biased agonist, and plays a role on switching cellular signalings mediated from EP4 receptors to EP2 receptors.
Therefore, we here examined if PGD_2 and these five metabolites act on DP receptors as biased agonists, and found out they showed different profiles in terms of DP receptor activities.
Thus, PGD_2 and PGJ_2 acted as full agonists with similar potencies, whereas, Δ^{12}-PGJ_2 acted as a partial agonist to the cAMP system and T cell factor/β-catenin transcriptional activity.
These results suggest that the metabolites of PGD_2 are not simply inactivated metabolites, but may have some physiological significance in the inflammatory response mediated by DP receptors.

1-P-094

Identifications of novel drugs of GIRK channel and its disease-causing mutants

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G-protein-gated inwardly rectifying K^+ (GIRK) channels control various physiological functions. For example, GIRK1/2 heterotetramers in the brain regulate neuronal excitability; GIRK1/4 heterotetramers in the heart regulate heart rate. GIRK channels are potential therapeutic targets for the treatment of several diseases, such as atrial fibrillation and addiction. In the present study, we aim to identify novel agonists/antagonists of GIRK channel and its disease-causing mutants. By electrophysiological recordings using *Xenopus* oocytes expressing different GIRK subunits without G-protein-coupled receptors, we screened the effect of a chemical library containing hundreds of natural products on GIRK currents. We observed that some plant alkaloids inhibit the current of wild-type GIRK1/2 and GIRK1/4 channels and some other plant alkaloids strongly inhibit a neurologic disorder-causing mutant of GIRK2 channel, G156S, which locates at the selectivity filter and induces the loss of K^+ selectivity. Our data provided us with a clue toward the elucidation of the potential of natural products as sources of novel therapeutic agents on GIRK-targeted disease.

1-P-095

Development of anti-mouse CC chemokine receptor 8 monoclonal antibody C_8Mab-2

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CC motif chemokine receptor 8 (CCR8), a G protein-coupled receptor (GPCR), is highly expressed in regulatory T cells, T helper 2 cells, and cancer cells. It plays important role in allergic inflammation and cancer development. Therefore, specific monoclonal antibodies (mAbs) for CCR8 would be useful for diagnostic and therapeutic purposes of the diseases. However, the production of mAbs for GPCRs has remained very difficult. We have developed a novel method for the development of mAbs, named the Cell-Based Immunization and Screening (CBIS) method. In the present study, an SD rat was immunized with mouse CCR8-overexpressed CHO-K1 cells (CHO/mCCR8). The hybridomas expressing anti-mCCR8 mAbs were screened by using CHO/mCCR8 and CHO-K1 cells. We obtained 73 strongly anti-mCCR8 mAb-expressing hybridomas from 1,916 hybridomas, and we finally established C_8Mab-2 (IgG_{2b}, kappa). C_8Mab-2 selectively reacted to CHO/mCCR8 cells in a dose-dependent manner, but not to CHO-K1 cells, in flow cytometry. C_8Mab-2 also recognized endogenous mCCR8 in a mouse lymphocyte-like cell line (P388) and a mouse macrophage-like cell line (J774-1). Furthermore, C_8Mab-2 visualized mCCR8 in CHO/mCCR8, P388, and J774-1 cells in immunocytochemistry. In conclusion, we developed the anti-mCCR8 mAb, C_8Mab-2, which is available for detecting endogenous and exogenous mCCR8 in flow cytometry and immunocytochemistry. C_8Mab-2 would be usable for diagnosis and medical treatment for allergic inflammation and cancer in mouse models.

1-P-096

A newly discovered MRGPRA3 agonist by high-throughput screening induces scratching behaviors

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The Mas-related receptor A3 (MRGPRA3), an orphan GPCR, is expressed specifically in the dorsal root ganglion (DRG) sensory neurons, and it has recently been attracting much attention as an itch inducer. While MRGPRA3 responds to chloroquine which is an anti-malaria drug and causes strong itch, chloroquine requires high concentrations to activate MRGPRA3 and also produces MRGPRA3-independent responses. Therefore, in order to unveil the role of MRGPRA3, a new tool that enables selective manipulation of MRGPRA3 function is needed. In this study, we screened a series of small-molecule chemical libraries (1,084 compounds) to explore agonists for MRGPRA3 by high-throughput Ca^{2+} imaging. We identified that papaverine, an opium alkaloid, specifically evoked Ca^{2+} responses in cells expressing MRGPRA3 without affecting responses of other MRGPRs subtypes. Papaverine evoked Ca^{2+} responses in a subpopulation of DRG neurons that responded to chloroquine. In addition, we found that intradermal injection of papaverine to the cheek produced scratching behavior in a histamine-independent manner and did not produce nociceptive wiping behavior. Furthermore, the papaverine-induced scratching behavior was suppressed by a selective ablation of MRGPRA3-expressing primary afferent neurons or a genetic knockout of gastrin-releasing peptide receptors (GRPR: a crucial receptor for spinal itch transmission). Taken together, these results uncover a new pharmacological action of papaverine that is a potent and selective agonistic effect for MRGPRA3, which could be a powerful tool for research investigating the biological role of MRGPRA3 and the physiology and pathology of itch.

1-P-097

Inhibitory effects of CRAC channel current by anti-depressant

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Activation of glial cells contributes neural disorders like depression and neuropathic pain through the release of nitric oxide and cytokines. The release depends on intracellular Ca2+ levels. One of the Ca2+ entry pathways is Ca2+ release-activated Ca2+ (CRAC) channel. Anti-depressants are used for treatment of trigeminal neuralgia, but the effects of anti-depressants on CRAC channel have remained unsolved. We therefore examined the inhibitory effects of anti-depressants on the CRAC channel using patch-clamp technique. We recorded the CRAC channel current in rat basophil leukemia (RBL) cells instead of glial cells. The reasons are 1) RBL cells are model cells to record the CRAC channel current, and 2) glial cells have many kinds of ion channels. The CRAC channel was activated by adding 10-mM IP3 in the pipette solution. Membrane potential was held at 0 mV and was changed every 3 s from -120 mV to 80 mV with the 50-ms ramp wave. The membrane current showed the inward rectification, and its polarity reversed at >50 mV. These properties are hallmarks of the CRAC channel. Duloxetine, one of the anti-depressants, inhibited the CRAC channel current at -100 mV in a time-dependent and a concentration-dependent manners. The rank order of the inhibition was duloxetine = paroxetine > nortriptyline > imipramine. Blockade of the CRAC channel might be one of the pain relief mechanisms of duloxetine.