3-P-188

A novel neuro-vascular culture system revealed that the damage of vascular cells by Alzheimer's amyloid-β assemblies, amylospheroids, increases the expression of BACE protein through endothelial cells-derived angiotensin II release

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In Alzheimer's disease (AD), the presence of cerebrovascular dysfunction worsens amyloid-beta (Aβ) pathology in neurons, but the molecular mechanism remains unclear. We have previously shown that Aβ assemblies, amylospheroids (ASPD) (Noguchi et al. *JBC* 2009), are present on blood vessels of AD brains and suppress the activity of endothelial NO synthase by binding to the α3 subunit of Na^+ pump on vascular endothelial cells (Sasahara et al. *iScience* 2021). This suggested the possibility that ASPD-induced endothelial dysfunction may lead to worsening the Aβ pathology. To verify this, we newly constructed a tri-culture system using cerebrovascular endothelial cells, pericytes, and cerebral cortical neurons (reported at the last Annual Meeting). The addition of ASPD into the culture medium on the vascular cell side of the tri-culture system increased the expression of BACE protein in neurons. Interestingly, this increase was not observed in the system excluded pericytes. Preliminary data suggested that angiotensin II released from endothelial cells may play a role in the increase of BACE through the angiotensin AT2 receptor-bradykinin B2 receptor pathway in neurons. These findings suggest a new molecular mechanism between cerebrovascular dysfunction and the worsening of neuronal Aβ pathology.

3-P-189

Hypercholesterolemia may play protectively against cerebral aneurysms

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[Introduction] subarachnoid hemorrhage (SAH) is a life-threatening stroke and can be mainly caused by a ruptured aneurysm of cerebrovascular blood vessels. One-third of patients could survive with good recovery, one-third will survive with a disability, and one-third will die. It is well known that lowering blood cholesterol levels is mandatory in the prevention of cerebral circulatory disorders. However, the relationship between cholesterol and cerebral aneurysm is still controversial. In this study, we elucidate the above relationship by monitoring aneurysm and SAH in an aneurysm model of LDL receptor/ Apobec 1 double knock out ($LA^{-/-}$) mice and that of control mice.

[Method] Hashimoto model of animal cerebral aneurysms was performed.

Briefly, the left kidney was excised one week before the experiment. Elastase was administered to the subarachnoid space to damage the cerebral artery and sustained-release deoxycorticosterone was placed subcutaneously.

[Results] 1)The rupture rate of cerebral aneurysms was significantly lower in the $LA^{-/-}$ group than in the control group. 2) The collagen layer of the cerebral vessels was sturdier in $LA^{-/-}$ mice than control mice at one week after the induced aneurysm model. 3) However there is no difference in cerebral vessels including the collagen layer between LA-/- group and the control group in the intact condition.

[Conclusion] In this study, we identified the relationship between cholesterol level and subarachnoid hemorrhage. It will be guessed the transformation of the cerebral vessels had occurred after the aneurysm induction.

3-P-190

Analysis of pericytes derived from the stroke-prone spontaneously hypertensive rats

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Brain pericytes play an important role in the stabilization of vascular formation, maintaining the BBB function, regulating the blood flow synergistic with neuronal activation, and inflammatory processes. Hence, pericytes dysfunction may lead to numerous CNS diseases including neurodegeneration and cerebrovascular diseases. The aim of this study was to elucidate the role of pericytes in cerebrovascular disease. We isolated pericytes from Stroke-prone spontaneously hypertensive rat (SHRSP), which is accepted as an animal model of hypertension and cerebrovascular disease, and normotensive control rat (WKY). To reveal the role of pericytes on the function of the blood-brain barrier (BBB), we constructed an in vitro BBB model using brain endothelial cells (BECs) and pericytes. The effect of pericyte on the barrier function of BECs was similar between SHRSP and WKY, but the time course was different. We also performed the comparison of gene expression in pericytes between SHRSP and WKY. Expression levels of several genes in SHRSP were different from that of WKY. Especially, analysis of gene expression comparison indicated that calcium responses might be changed in SHRSP. SHRSP pericytes might be involved in occurs BBB dysfunction even if chronic hypertension and onset of stroke are not well-established.

3-P-191

Suppressive effects of cervical lymph nodes excision on pathology of ischemic cerebral stroke

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Introduction: Reduced cerebral blood flow damages the brain tissue in ischemic stroke. Recently, some studies have been reported the involvement of peripheral immune cells in the stroke pathology. Whereas, the existence of the meningeal lymphatic vessels was confirmed in the brain, suggesting that the peripheral immune cells enter the brain through the cervical lymph nodes (CLNs). In the present study, to elucidate a role of the CLNs in the stroke pathology, we evaluated the effects of surgical excision of the CLNs against the stroke.
Method: The transient middle cerebral artery occlusion (tMCAO) model mice were surgically prepared as ischemic cerebral stroke model by inserting a filament on male C57BL/6J mice at 8-9 weeks-old. Cervical lymphadenectomy (CLNE) was microscopically conducted immediately before tMCAO. Motor coordination was evaluated by beam test.
Results: Cerebroventricular injection of Evans blue stained the CLNs, confirming the direct connection between the CLNs and the brains. Motor coordination was impaired three days after tMCAO, and the dysfunction was suppressed by excision of the CLNs.
Conclusion: Motor function impaired in tMCAO model mice were improved by removing the CLNs, which suggests that CLNs are involved in the pathology of ischemic cerebral stroke including motor dysfunction.

3-P-192

Ischemia-induced Zn^{2+} accumulation is modulated by EAAT3 diurnal fluctuation in the hippocampus

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[AIM] Stroke during sleep shows a worse prognosis than that during wakefulness, but the mechanism is mostly unknown. In ischemic hippocampus, extracellular Zn^{2+} accumulates in neurons, resulting in neuron death. Excitatory amino acid transporter 3 (EAAT3) is involved in Zn^{2+} homeostasis. Here, we investigated whether diurnal variations in ischemic injury is mediated by altered Zn^{2+} accumulation via EAAT3.
[METHODS] Mice (12 weeks old) were subjected to ischemia/reperfusion (I/R) at 09:00 (ZT4) or 23:00 (ZT18). At 72 h after I/R, Zn^{2+} accumulation and neuron death were assessed by the Zn^{2+}-specific probe, TSQ, and Fluoro-Jade B (FJB), respectively. Next, mice were subjected to I/R at ZT18 after injection (ICV) with a non-selective EAAT3 inhibitor (TBOA, 12.5 mM, 2 mL) and then Zn^{2+} accumulation and neuron death were evaluated as same as above. EAAT3 expression and glutathione (GSH) level were detected by western blot and GS-NEM, respectively.
[RESULTS] I/R-induced TSQ (+) and FJB (+) cells were less at ZT18 than ZT4. TBOA increased TSQ (+) and FJB (+) cells. Besides, hippocampal EAAT3 expression and GSH level were higher at ZT18 than ZT4.
[CONCLUSION] These results suggest I/R-induced Zn^{2+} accumulation displays temporal changes via diurnal variations in EAAT3 expression, which affects susceptibility to hippocampal ischemic injury.

3-P-193

Brain pericytes promote brain endothelial DHA uptake through inducing membrane expression of the transporter MFSD2A

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Blood-brain barrier (BBB), which is formed by brain microvessel endothelial cells (BMECs), astrocytes and brain pericytes, selectively transports substances in the blood. Nutrients are actively taken up by the brain through various transporters on BMECs. In particular, docosahexaenoic acid (DHA) is most abundant nutrient in the brain and important for many neurophysiological functions. A major transporter for DHA at the BBB is the major facilitator superfamily domain containing protein 2a (MFSD2A), which is expressed exclusively in BMECs. Although brain pericytes regulate expression of MFSD2A on BMECs, its mechanism remains unclear. Here, we used non-contact cocultured BBB models consisting of primary cultures of rat brain endothelial cells (RBECs) and rat brain pericytes. After 1 and 3 days of coculture, expression levels of MFSD2A were evaluated by western blot analysis and immunofluorescent staining. The impact of brain pericytes on endothelial DHA uptake was evaluated by [14C] DHA radioactivity in RBECs cocultured with rat brain pericytes. In RBECs cocultured with rat brain pericyte for 3 days, MFSD2A protein expression in cell lysate and plasma membrane was significantly increased compared with RBEC monolayer. Brain pericytes significantly increased uptake of [14C] DHA by RBECs. Our findings suggested that brain pericytes promote DHA uptake by BMECs through increasing MFSD2A expression on plasma membrane of BMECs.

3-P-194

(+)-Hopeaphenol inhibits Poly IC-induced innate immunity activation via IFN-β in human cerebral microvascular endothelial cells

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The drug development for regulating the excess innate immune reactions is important to prevent inflammatory diseases. Resveratrol is a structural unit of its oligomers and has various neuroprotective effects such as the inhibition of chemokine expression. The aim of our study is to investigate the effects of resveratrol oligomers on CXCL10 expression, a chemoattractant of lymphocytes into brain. We examined the effects of (+)-hopeaphenol, a resveratrol tetramer, and its derivatives on CXCL10 expression induced by polyinosinic–polycytidylic acid (poly IC; a synthetic analog of dsRNA as a Toll-like receptor 3 ligand) in cultured human cerebral microvascular endothelial cell line. Only (+)-hopeaphenol (1-10 μM) inhibited poly IC-induced CXCL10 expression in a dose-dependent manner. We further confirmed that (+)-hopeaphenol significantly reduced poly IC-induced expression of IFN-β, an upregulator of CXCL10. Phosphorylation of NF-κB, but not of interferon regulatory factor 3, was inhibited by (+)-hopeaphenol. These results suggest that (+)-hopeaphenol can negatively regulates poly IC-induced NF-κB/IFN-β/CXCL10 axis, and may be effective to prevent inflammatory diseases associated with excess innate immunity.

3-P-195

Effects of mizagliflozin, a selective SGLT1 inhibitor, on chronic hypoperfusion-induced vascular cognitive impairment.

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Introduction: Sodium/glucose cotransporter 1 (SGLT1) participates in ischemia-reperfusion-induced neural injury and the development of vascular cognitive impairment. However, whether mizagliflozin, a selective SGLT1 inhibitor, can improve the vascular cognitive impairment still unknown. We examined the effects of mizagliflozin on vascular cognitive impairment in mice.

Methods: Cerebrovascular hypoperfusion was created using a mouse model of asymmetric common carotid artery surgery (ACAS). Two and/or 4 weeks after ACAS, all experiments were performed.

Results: Cerebral blood flow (CBF) was decreased in ACAS compared with sham-operated mice. Mizagliflozin did not reverse the decreased CBF of ACAS mice. Mizagliflozin reversed the ACAS-induced decrease in the latency to fall in a wire hang test of ACAS mice. Moreover, it also reversed the ACAS-induced longer escape latencies in the Morris water maze test of ACAS mice. ACAS increased SGLT1 and interleukin 1β (IL-1β) gene expressions in mouse brains and mizagliflozin did not normalize these gene expressions in ACAS mice. Hematoxylin/eosin staining demonstrated that pyknotic cell death in the ACAS mouse hippocampus was improved in mizagliflozin-treated ACAS mice . In PC12HS cells, IL-1β increased SGLT1 gene expression and decreased survival rates of cells. Mizagliflozin increased the survival rates of IL-1β-treated PC12HS cells.

Conclusions: These results suggest that mizagliflozin can improve cerebral hypoperfusion-induced vascular cognitive impairment through the inhibition of neural SGLT1.

3-P-196

Establishment of a mouse model of spinocerebellar ataxia type 34

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Spinocerebellar ataxia type 34 (SCA34) is an autosomal dominant neurodegenerative disease characterized by slowly progressive ataxia. It is caused by missense mutations of elongation of very long-chain fatty acids-4 (ELOVL4), which is mainly expressed in neurons in the cerebellum. In this study, we attempted to establish SCA34 model mice via the gene transfer of mutant ELOVL4 to cerebellar neurons and to reveal the pathogenic mechanisms of SCA34. We used adeno-associated viral (AAV) vectors to express wild-type or missense mutant (W246G) FLAG-ELOVL4 in a neuron-specific manner. These AAV vectors were injected into the cerebella of 4-week-old male ICR mice. Motor function was evaluated by beam-walking tests. Mice expressing mutant FLAG-ELOVL4 showed progressive motor impairment from the 2nd week after the AAV vector injection. Immunohistochemical analyses of cerebellar sections revealed that the expression of mutant FLAG-ELOVL4 triggered a slight loss of Purkinje cells and microglial activation at an early phase. The severe degeneration of Purkinje cells, shrinkage of the molecular layer, and activation of both microglia and astrocytes were observed in cerebellar slices of mice expressing mutant FLAG-ELOVL4 at the late phase. Taken together, we succeeded in establishing a mouse model of SCA34.

3-P-197

Involvement of P-glycoprotein in α-Synuclein aggregate-induced inflammatory mediator release by brain pericytes

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The pathological hallmark of Parkinson disease (PD) is a widespread distribution of the aggregated α-synuclein (α-Syn) proteins in the inclusions known as Lewy bodies. Exogenous α-Syn secreted from neurons could induce neuroinflammation. We previously reported that pericytes, an essential component of the blood-brain barrier (BBB), released various inflammatory mediators in response to monomeric α-Syn through Toll-like receptor (TLR) 4. Here, we investigated whether α-Syn aggregates also induced the release of inflammatory mediators from pericytes. Then, we intended to characterize the α-Syn-activated pericytes. In response to α-Syn aggregates, pericytes released higher levels of IL-1β, IL-6, MCP-1, TNF-α and MMP-9 than the other cell types of the BBB (brain endothelial cells and astrocytes). TAK242 (a TLR4 inhibitor, 5 μM) inhibited the release of these mediators induced by a 24-hr exposure of α-Syn aggregates (5 μg/mL). P-glycoprotein (P-gp) expression in pericytes was increased by α-Syn aggregates concurrently with the release of inflammatory mediators. TAK242 also inhibited the α-Syn aggregate-induced increase in P-gp expression in pericytes. These results suggest that the increased P-gp expression in pericytes may characterize the α-Syn-activated pericytes and is involved in the release of inflammatory mediators from pericytes.

3-P-198

Cyclophillin A is involved in the uptake of oligomericα-synuclein by brain pericytes

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Parkinson's disease (PD) is characterized by widespread distribution of Lewy bodies, which are mainly composed of aggregated α-synuclein(α-Syn), in the brain. The ability of glial cells to take up extracellular α-Syn contributes to the wide distribution and accumulation of α-Syn in the brain. However, it is unclear whether α-Syn is taken up by brain pericytes, which are a constituent cell of the cerebral microvasculature. We previously found that α-Syn monomer was taken up by brain pericytes. In this study, we investigated the intracellular uptake mechanism in pericytes for more toxic α-Syn oligomers. We used primary cultures of rat brain pericytes. Pericytes took up α-Syn oligomers, and the uptake was decreased at low temperature (4 ℃). After a 1-hr incubation of a-Syn oligomer, the intracellular α-Syn oligomer in pericytes was time-dependently decreased during the next 6 hr. Furthermore, cyclosporin A(CsA), a P-glycoprotein (P-gp) inhibitor, increased the uptake of α-Syn oligomer by pericytes. However, siRNA-mediated knockdown of P-gp failed to increase the uptake of α-Syn oligomers by pericytes. Knockdown of Cyclophillin A (CypA), a molecular target of CsA to inhibit calcineurin activity, decreased the uptake of α-Syn oligomer by pericytes. These results suggested that α-Syn oligomer uptake by pericytes is energy- and CypA-dependent.

3-P-199

Fosl1 gene expression in the mouse hippocampus of earlier stages of epileptogenic processes

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We recently reported that LPS markedly increased *Fosl1* (*Fra1*, one of Fos family) expression, and knockdown of *Fosl1* significantly suppressed the expression of inflammatory cytokines in murine BV-2 microglial cell lines. These observations suggest that *Fosl1* is involved in microglial inflammatory activation. Our previous studies also showed that inflammation in the hippocampus at the early phase of the epileptogenic processes could induce spontaneous seizures in pilocarpine (PILO)-induced status epilepticus (SE) mouse model. Therefore, in this study we clarified relationship between *Fosl1* expression and inflammation in the hippocampus after PILO-SE. Real-time PCR analyses showed that expression of hippocampal *Fosl1* displayed more than a 100-fold increase on the 1st and 2nd days after PILO-SE, then gradually decreased and still high level (45-fold of control) on the 7th day. Next, in order to identify the cell types expressing *Fosl1*, neurons, glial cells (astroglia and microglia), and vascular endothelial cells were separated from hippocampus of PILO-SE and control mouse using a FACSAria II (BD). The expression analyses showed that hippocampal *Fosl1* was mainly expressed in the glial cells. The relationship between increased hippocampal *Fosl1* expression after PILO-SE and intrahippocampal inflammation is under investigation.

3-P-200

Dopamine decreases LPS-induced expression of proinflammatory cytokines in microglial cells through the formation of dopamine quinone in the mouse striatum.

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We previously reported that pretreatment with dopamine decreased lipopolysaccharide (LPS)-induced expression of proinflammatory cytokines through the formation of dopamine quinone in cultured microglial cells, and that loss of dopaminergic neurons induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) enhanced LPS-induced mRNA expression of proinflammatory cytokines in the mouse striatum. In this study, we examined the effect of L-dopa/carbidopa on the enhancement of LPS-induced mRNA expressions of proinflammatory cytokines by MPTP in the mouse striatum. The levels of cytokine mRNA and quinoprotein, an indicator of dopamine quinone formation, in the striatum were examined by real-time RT-PCR and NBT/glycinate assay, respectively. Co-administration of L-dopa (50 mg/kg) and carbidopa (5 mg/kg), once daily for 5 days, significantly attenuated the enhancement of LPS-induced increase in mRNA levels of TNF-α, IL-1β, and IL-6 by MPTP in the striatum. The decrease of quinoprotein level in the striatum by MPTP was also attenuated by the co-administration of L-dopa and carbidopa. These results suggest that dopamine decreases LPS-induced expression of proinflammatory cytokines in microglial cells through the formation of dopamine quinone in the mouse striatum.

3-P-201

PM226, a cannabinoid CB_2 receptor agonist, protects retinal ganglion cells against excitotoxicity in the mice

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Excitotoxicity is thought to be involved in the neuronal cell death induced by glaucoma. It has been reported that cannabinoid CB_2 receptor stimulation protects against light-induced retinal injury and tunicamycin-induce photoreceptor cell injury. In the present study, we examined whether PM226, a cannabinoid CB_2 receptor agonist, protected retinal ganglion cells against the NMDA-induced neurotoxicity in the mice, *in vivo*. Male ICR mice of 8-12 weeks old were subjected to intravitreal NMDA (40 nmol/eye). PM226 (10~100 pmol/eye) was intravitreally injected simultaneously with NMDA. Eyes were enucleated 7 days after NMDA injection, and the whole mount retinas were prepared. Immunohistochemistry using Alexa Fluor 488-conjugated anti-NeuN antibody was carried out to visualize retinal ganglion cells in the whole mount retina. PM226 (100 pmol/eye) significantly reduced retinal ganglion cell loss 7 days after NMDA injection. These results suggest that PM226 protects retinal ganglion cells against excitotoxicity, and that cannabinoid CB_2 receptor stimulation may be protective against excitotoxicity in the mice retina.

3-P-202

Insulin promotes expression of *MIDN*, a novel genetic risk factor for Parkinson's Disease, mediated through AP-1

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Previously, we demonstrated that *Midnolin* (*MIDN*) is a novel genetic risk factor for Parkinson's disease in Yamagata and British population by genome-wide association study. In PC12 cells, neurite outgrowth was completely blocked by *Midn* deletion by genome-editing. In this study, because we found that insulin promoted *MIDN* gene expression in SH-SY5Y human neuroblastoma cells, we attempted to clarify the mechanism in detail. *MIDN* expression was promoted accompanied by the activation of ERK1/2 and PI3-kinase. We also used a DNA construct encoding human *MIDN* promoter linked to firefly *luciferase*, and identified important regions on the *MIDN* promoter. The region (-121 bp~-99 bp) includes TFAP2 consensus sequences, *MIDN* promoter activity was enhanced by mutation at the sequences and overexpression of dominant-negative mutant of TFAP2. In contrast, the region (-71 bp~-57 bp) includes AP-1 consensus sequence, *MIDN* promoter activity was decreased by mutation at the sequence. Insulin promoted AP-1 activity accompanied by the expression of c-FOS, which was blocked by U0126 and wortmannin, an ERK1/2 and PI3-kinase inhibitor, respectively. *MIDN* promoter activity was decreased by decoy oligodeoxynucleotide for AP-1 and AP-1 inhibitor, SR11302. These results suggest that insulin promotes *MIDN* gene expression mediated through AP-1.

3-P-203

Analysis of the dynamics of primary cilia in a humanized APP mouse model

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Primary cilium is a cellular protrusion appeared on most mammalian cell types. This organelle functions as a sensory structure receiving the extracellular milieu in response to environmental change. Neuronal ciliary membrane is highly enriched for receptors, in particular a set of certain GPCRs including melanin-concentrating hormone receptor 1 (MCHR1), determine a host of crucial physiologies. Disrupting cilia structure or function results in a spectrum of diseases collectively called ciliopathies. Common to human ciliopathies is cognitive impairment, a symptom also observed in Alzheimer's disease (AD). One hallmark of AD is accumulation of senile plaques composed of neurotoxic amyloid-β peptide, which is generated by the proteolytic cleavage of the amyloid precursor protein (APP). Here, we report that the hippocampal CA1 and the CA3 neuronal cells in $App^{NL\text{-}G\text{-}F}$ knock-in mice still had canonical cilia molecule adenylyl cyclase 3- and MCHR1-positive cilia but their length were significantly changed as compared to the corresponding wild-type mice. In addition, a significant difference of cilia prevalence between NLGF- and wild-mice also observed in selective hippocampal regions. There is growing evidence that primary cilia dynamics regulate synaptic connectivity and formation of neuronal dendrites. Thus, our observations raise the important possibility that structural and functional alterations in neuronal cilia might have a role in AD development.

3-P-204

The effect of ALS-related mutation on lipid binding of sigma-1 receptor and its relationship with agonist action

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Sigma-1 receptor (σ1R) is a chaperone protein localized in ER membrane. Intrinsic molecules like cholesterol and some clinical drugs are known to bind and regulate the receptor. In this decade, twelve mutations of σ1R were discovered in motor neuron diseases like ALS and dHMN. We previously reported that the mutant (E102Q) which was identified in ALS shows abnormal resistant to detergents, intracellular aggregation, and toxicity in NSC-34 cells, a motor neuron-like cultured cells. However, it is fully unknown how the mutation alters the feature of σ1R.

We transfected the cells with wildtype and the mutant and performed pulldown assay using cholesterol-beads. Cells were treated with the agonist SA4503 to investigate if the agonist changes the formation of σ1R and lipid complex. Moreover, cells were treated with BODIPY-Cholesterol to analyze cellular distribution of the lipid.

As a result, the mutant but not wildtype σ1R binds with cholesterol in NSC-34 cells. SA4503 inhibited the binding of the mutant and cholesterol. BODIPY-Cholesterol was accumulated in the mutant aggregation. These results suggest that ALS-related mutation causes σ1R to bind with cholesterol, leading to aberrant aggregations and toxicity. Moreover, modulations by the agonist can inhibit the binding with the lipid and aggregation.

3-P-205

5-Lipoxygenase inhibitors suppressed the neurotoxicity of secretory phospholipase A_2

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Mammalian secretory phospholipase A_2 ($sPLA_2$) is associated with neurological diseases including brain ischemia. Previously, we have reported the contribution of arachidonate (AA) metabolites via cyclooxygenase (COX) to the neurotoxicity of $sPLA_2$. However, it has not yet been sufficiently clarified how arachidonate metabolites via 5-lipoxygenase (LOX) is involved in the neurotoxicity of $sPLA_2$. In the present study, we evaluated protective effects of LOX inhibitors (phenidone and AA-868) against the group IB porcine $sPLA_2$ ($sPLA_2$-IB) in the primary culture of rat cortical neurons. $sPLA_2$-IB-induced neuronal apoptosis had been established as the *in vitro* model for cerebral ischemia. $sPLA_2$-IB generated reactive oxygen species and triggered the influx of Ca^{2+} into neurons via L-type voltage-dependent calcium channel (L-VDCC). We confirmed protective effects of a radical scavenger, vitamin E, and an L-VDCC blocker, nimodipine, on the neurotoxicity of $sPLA_2$-IB. Before neuronal cell death, the enzyme produced AA metabolites via COX and LOX, prostaglandin (PG) D_2 and leukotriene (LT) B_4, respectively. As well as COX inhibitors, LOX inhibitors prevented neurons from the $sPLA_2$-IB-induced cell death. A PGD_2 receptor antagonist, BWA868C, did not exhibit protective effect on the neurotoxicity of $sPLA_2$-IB. On the other hand, receptor antagonists for LTB_4 (LY293111) and cysteinyl LTs (ONO-1078) suppressed the neurotoxicity of $sPLA_2$-IB in a concentration-dependent manner. Furthermore, these LTR blockers ameliorated morphological alterations such as shrank neuronal cell bodies and shortened neurites in the $sPLA_2$-IB-treated neurons. Thus, the present study suggested that AA metabolites via LOX might be involved in the $sPLA_2$-IB-induced neuronal apoptosis.

3-P-206

Short-term intranasal administration of rotenone provides a useful mouse model with chemosensory and mild cognitive impairments.

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Parkinson's disease (PD) characteristically presents with multiple symptoms as well as distinctive motor impairments. Smell disorders, followed by mild cognitive impairment, frequently occur in the early stage of PD, and both are progressed as motor symptoms. To elucidate the mechanisms underlying these prodromal symptoms, the development of appropriate animal models is required. This study aimed to verify whether short-term intranasal administration of rotenone (Rot) causes these impairments in mice. Chemosensory/cognitive/motor function was assessed in 1-week Rot administrated-mice (1w-Rot) 1 week after finishing intranasal Rot administration. Motor function was also examined in 4-week Rot administrated-mice (4w-Rot) from 0 to 5 week after starting intranasal Rot administration. After behavioral test, the number of catecholamine neurons (CA-Nos) in the substantia nigra (SN) was examined. Intranasal Rot administration for 1 week simultaneously caused olfactory, taste, and taste memory impairments. Interestingly, motor deficits were induced from the third week after starting 4-week intranasal Rot administration. Despite no apparent change in SN CA-Nos of 1w-Rot, that of 4w-Rot was significantly reduced by 75% at the fifth week. These results suggest that 1w-Rot is useful as a mouse model for the early stage of PD.

3-P-207

Identification of a novel sleep-regulating factor by in vivo screening of neurodegenerative disease-related factors

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Neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease are known to be accompanied by sleep disorders (Raman, Sleep Med. Clin., 2018). Conversely, in recent years, the sleep disorders have been shown to be a risk factor for the neurodegenerative diseases (Sabia et al., Nat. Commun., 2021). Although these findings suggest a link between neurodegenerative diseases and sleep disorders, the molecular mechanisms involved here are yet to be elucidated. In this study, we addressed the molecular mechanisms that link these two processes by analyzing the contribution of neurodegenerative diseases-related factors to sleep regulation. By combining the adeno-associated viral vector which delivers genes throughout the whole mouse brain, and the Snappy Sleep Stager (SSS) method which measures sleep in a high-throughput manner from mouse respiratory pattern, we analyzed the effects of more than 50 neurodegenerative disease-related factors and their mutants on sleep. As a result, we have identified a novel sleep-regulating factor that can alter sleep duration in mice significantly. The factor might provide insights into the molecular basis linking sleep regulation and pathogenesis of neurodegenerative diseases.

3-P-208

Role of noradrenergic transmission within the ventral bed nucleus of the stria terminalis in nicotine withdrawal-induced aversive behavior

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Cessation of smoking leads to nicotine withdrawal symptoms such as anxiety, depression, and dysphoria, which could lead to smoking relapse. Therefore, it is necessary to clarify the neural mechanisms of nicotine withdrawal-induced negative emotions. We have previously reported that noradrenergic transmission in the ventral bed nucleus of the stria terminalis (vBNST) plays a crucial role in pain-induced aversion. To investigate the role of intra-vBNST noradrenergic transmission in nicotine withdrawal-induced aversion, a conditioned place aversion (CPA) test and in vivo microdialysis were conducted. Male Sprague-Dawley rats were used. Each animal was subcutaneously implanted with a nicotine- or saline-filled osmotic pump on day 1. Nicotine was continuously administered at 4.8 mg/kg/day. Nicotine withdrawal was precipitated by administration of mecamylamine (3.0 mg/kg) on day 15. Noradrenaline release in the vBNST was increased after mecamylamine administration in the nicotine group, but not in the saline group. Nicotine withdrawal induced aversive behavior, which was attenuated by intra-vBNST injection of timolol, a β-adrenoceptor antagonist. These results suggest that enhanced noradrenergic transmission in the vBNST during nicotine withdrawal plays an important role in nicotine withdrawal-induced aversive behavior.

3-P-209

Effects of the cannabinoid CB_1 receptor antagonist AM251 on sensorimotor gating deficits during withdrawal in mice repeatedly administered with methamphetamine

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Repeated administration of methamphetamine causes sensorimotor gating deficits in experimental animals and human. However, the withdrawal effects after the repeated administration of methamphetamine have been still unclear. In this study, we investigated the involvement of cannabinoid CB_1 receptors on impairment of sensorimotor gating function during withdrawal in mice repeatedly administered with methamphetamine.

Mice were subcutaneously administered methamphetamine (1.8 mg/kg), every other day for 30 days. After methamphetamine withdrawal, the mice were tested a sensorimotor gating function by the prepulse inhibition test. AM251 (3.2 mg/kg, s.c.), a CB_1 receptor antagonist, was administrated in mice repeatedly administered with methamphetamine.

Prepulse inhibition was significantly suppressed during withdrawal after repeated methamphetamine. This impairment of prepulse inhibition was ameliorated by AM251 in combination with repeated methamphetamine and also by AM251 during withdrawal after repeated methamphetamine. However, singly administration of AM251 before the test could not altered the prepulse inhibition deficits.

These findings suggest that persistent activation of the CB_1 receptors could lead to the development of sensorimotor gating deficits during withdrawal after repeated methamphetamine administration.

3-P-210

Changes in miR-7020-5p expression in methamphetamine-induced rewarding effect

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Methamphetamine is one of the most abused drug in Japan and produces a strong rewarding effect. However, little is known about the mechanisms underlying methamphetamine-induced rewarding effect. Recent studies demonstrated that microRNA have important role in the regulation of several physiological functions. In the present study, we investigated the expression of microRNAs in methamphetamine-induced rewarding effect. The rewarding effect was evaluated by conditioned place preference. Methamphetamine (1mg/kg, s.c.) produced a significant rewarding effect. The mice were killed by decapitation and the limbic forebrain (containing nucleus accumbens) was dissected. Comprehensive analysis of microRNA expression in methamphetamine-induced rewarding effect was performed by microRNA array. The microRNA array analysis showed 20 significant changed microRNAs in the methamphetamine-induced rewarding effect. Especially, miR-7020-5p highly upregulated (fold change >30) in methamphetamine-induced rewarding effect. We next investigated the target scan for miR-7020-5p by database scan. We detect transient receptor protein subfamily C member 4 associated protein as a candidate gene. In conclusion, our findings suggest that the upregulation of miR-7020-5p may associate with the development of methamphetamine-induced rewarding effect.

3-P-211

Effects of pretreatment with a glycogen synthase kinase-3 inhibitor on methamphetamine-induced hyperlocomotion, stereotypy and reward in mice

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Glycogen synthase kinase-3 (GSK-3) is one of the most essential serine/threonine kinases, which are constitutively active, multifaceted, and ubiquitous in nature. In mammalian cells, GSK-3 is formed as the two isoforms termed GSK -3alpha and GSK-3beta. GSK-3beta is present in a high concentration in the abundance of tissues in the central nervous system, regulating a crucial role in neuronal signaling pathways. The research for involvement of GSK-3beta signaling in drug abuse liability has been progressed based on the studies investigating molecular and cellular mechanism of action, but few reports have been made on animal research so far. In this presentation, we will demonstrate that pretreatment with SB216763 (2.5 mg/kg), a GSK-3beta inhibitor, had no effects on methamphetamine (METH)-induced hyperlocomotion (3 mg/kg of METH), stereotyped behavior (10 mg/kg of METH), and rewarding property (0.5 mg/kg of METH) in mice. These observations are completely different results from those of morphine-induced Straub's tail response (STR): pretreatment of mice with SB216763 significantly inhibited 30 mg/kg morphine-induced STR and attenuated the time of STR duration. Horizontal locomotor activity was also attenuated by SB216763.

3-P-212

Glycogen synthase kinase-3 inhibitors suppress morphine-induced Straub's tail reaction via a centrally acting mechanism

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Background: In the previous meeting, we have demonstrated that pretreatment with Glycogen synthase kinase-3 inhibitors (GSK3i) suppress morphine-induced Straub's tail reaction (STR). In this presentation, we investigated the GSK3i mechanism of action. Methods: In this presentation, expression levels of GSK3beta and phosphorylated GSK3beta (pGSK3beta^{ser9}) were examined in the region of the striatum and nucleus accumbens and in the cerebral cortex after systemic morphine. In constipated mice after morphine, the effect of GSK3i on gastrointestinal transit was also examined to reveal whether the action of GSK3i on morphine effects was central and/or peripheral. Results: Pretreatment of mice with SB216763 (5 mg/kg, s.c.) or AR-A014418 (3 mg/kg, s.c.) significantly inhibited morphine-induced STR. In the brain regions tested, expression levels of pGSK3beta^{ser9} in mice after morphine were not altered in mice treated with GSK3i in combination with morphine. The pretreatment with Gsk3i did not improve constipation in morphine-injected mice. Conclusions: The inhibitory effect of GSK3i on morphine-induced STR might not depend on the blockade of GSK3 in terms of phosphorylation levels of pGSK3beta^{ser9}. The mechanism of action is likely to be central but not peripheral.

3-P-213

Suppressive effects of glatiramer acetate on the addiction induced by methamphetamine via upregulation of osteopontin in mice

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【Introduction】

Methamphetamine (METH) is addictive drugs widely distributed in the world. Since METH users cannot be recovered from addictive symptoms, a novel medication is required. We have reported that local injection of osteopontin (OPN), an extracellular matrix protein, to the nucleus accumbens (NAc) suppresses behavioral alterations induced by METH in mice. Glatiramer acetate (GA, Copaxone®), a therapeutic reagent for multiple sclerosis, was reported to induce OPN in the brain. Here, we examined the therapeutic effects of GA on METH-induced behavioral alterations in mice.

【Methods】

C57BL/6J (8-9 weeks, male) received subcutaneous administration of GA (0.1 mg/mouse) and METH (1 mg/kg). After daily administration of GA for 14 days, behavioral analyses were conducted under METH conditioning. The brains were collected for immunohistochemistry (IHC) or flow cytometry (FCM).

【Results】

Pre-administration of GA to mice suppressed conditioned place preference, not hyperlocomotion, under METH conditioning. IHC and FCM revealed that OPN expression in the brain was upregulated in the microglia and macrophage.

【Conclusion】

These indicate that GA suppresses METH-preference behavior, possibly mediated by the upregulation of osteopontin. Taken together, GA is indicated to have a therapeutic potential for METH-preference behavior.

3-P-214

Distinct role of dopamine in the PFC and NAc during exposure to cocaine-associated cues

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The role of dopamine in the recognition of drug-associated environmental stimuli, retrieval of drug-associated memory and drug-seeking behaviors is not fully understood. Roles of dopamine neurotransmission in the prefrontal cortex (PFC) and nucleus accumbens (NAc) in the cocaine conditioned place preference (CPP) paradigm were evaluated using *in vivo* microdialysis. In mice that had acquired cocaine CPP, dopamine levels in the PFC, but not in the NAc, increased in response to cocaine-associated cues when mice were placed in the cocaine-chamber of the two-separated chambers. The induction of the dopamine response and the development of cocaine CPP were mediated through activation of glutamate NMDA/AMPA receptor signaling in the PFC during conditioning. Activation of dopamine D1 or D2 receptor signaling in the PFC was required for cocaine-induced locomotion, but not for the induction of the dopamine response or the development of cocaine CPP. Interestingly, dopamine levels in the NAc increased in response to cocaine-associated cues when mice were placed at the center of the two-connected chambers, which requires motivated exploration associated with cocaine reward. Dopamine neurotransmission in the PFC is activated by the exposure to the cocaine-associated cues, whereas dopamine neurotransmission in the NAc is activated in the process of motivated exploration of cues associated with cocaine reward. Furthermore, the glutamate signaling cascade in the PFC is suggested to be a potential therapeutic target to prevent the progression of drug addiction.

3-P-215

Cognitive dysfunction and hippocampal synaptic impairment induced by methamphetamine locally injection into the nucleus accumbens

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[Introduction] Methamphetamine (METH) is one of the most widely addictive drugs in the world. Repeated drug use frequently leads to cognitive impairment, but no effective treatment has been established. In the presents study, we examined the cognitive function of mice with injection of METH to the nucleus accumbens (NAc) to clarify the mechanism of cognitive impairment induced by METH.
[Method] C57BL/6J mice (8-week-old, male) received administration of METH *via* implanted cannula in the NAc for seven consecutive days. Behavioral experiments were performed to evaluate cognitive functions. To examine long-term potentiation (LTP), excitatory postsynaptic potential on Schaffer collateral in response to theta burst stimulation on CA1 was recorded on the acute hippocampal slices. Behavioral and electrophysiological experiments were performed one day after the last injection. Slices after stimulation were applied to Western blotto analyze phosphorylation of AMPA receptor (Ser831, Ser845).
[Results] Mice with METH in the NAc showed cognitive impairment in the novel object recognition test. LTP and phosphorylation of AMPA receptors were reduced in mice treated with METH.
[Conclusion] Local METH administration in the NAc is suggested to suppress hippocampal synaptic transmission to cause cognitive dysfunction.

3-P-216

Construction of the ELISA assay to quantify Semaphorin 3A in the adult brain

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Extracellular soluble signals that control several aspects of neuronal development are known to play a critical role in maintaining neuronal function and homeostasis in the mature nervous system. Abnormal expression and/or secretion of these molecules are therefore thought to be associated with the onset of various types of neurological disorders. It has been reported that the expression of Semaphorin 3A (Sema3A), a secreted type of repulsive axon guidance molecule, is impaired in several neurodegenerative disorders. However, due to the lack of a reliable Sema3A antibody, our knowledge about Sema3A expression in the adult brain is still limited. Here we report the identification of a pair of Sema3A monoclonal antibodies for the sandwich ELISA assay using the Autonomously Diversifying Library system. This ELISA assay recognized recombinant human Sema3A in the range of 0-100 pM, but not Sema3F, one of a subfamily of class III semaphorins. The specificity of this assay was also confirmed by using the embryonic brains from *sema3A* deficient mice as a negative control. Moreover, this assay could measure Sema3A concentration in TBS- and RIPA-soluble lysate obtained from the adult mice brains. These data suggested that our ELISA assay is a reliable tool for the validation of Sema3A as a biomarker in neurodegenerative disorders.

3-P-217

Acute intracerebroventricular injection of an eEF2K inhibitor lowers systemic blood pressure in hypertensive rats

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Eukaryotic elongation factor (eEF2) kinase (eEF2K) that phosphorylates a specific substate, eEF2, suppresses protein translation. We previously demonstrated that acute intravenous injection of a selective eEF2K inhibitor, A484954, lowered blood pressure (BP) via affecting peripheral vasculature in spontaneously hypertensive rats (SHR). It is recently recognized that the dysfunction of release and sensitivity of neurotransmitters in brain mediates systemic hypertension. In the present study, in order to test the hypothesis that eEF2K in brain is involved in an increase in BP of SHR, we examined the effects of acute intracerebroventricular (i.c.v.) injection of A484954 on BP in SHR. A484954 lowered mean BP, suggesting that eEF2K in the brain might be involved in the increase in systemic BP in SHR.

3-P-218

Cyclosporin A stimulates brain pericytes to induce inflammatory mediators related to blood-brain barrier dysfunction

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Cyclosporin A (CsA) is an immunosuppressive drug used for organ transplant patients. Increased brain transport of CsA elevates the risk for the occurrence of neurotoxic adverse effects including seizures under the pathological conditions. However, it has not been well known which cell types composing brain tissue is the target for the occurrence of CsA-induced neurotoxicity. Brain pericytes located in brain microvessels is a key player in the development of neuroinflammation and blood-brain barrier (BBB) dysfunction under the pathological conditions, which implicated in brain dysfunction including seizures characterized by neuronal hyperexcitability. We therefore hypothesized that CsA stimulates brain pericytes to release mediators associated with BBB dysfunction and neuroinflammation. In this study, brain pericytes obtained from rat brains were incubated with CsA (1-10 μM) for 24 h. CsA treatment significantly increased the release of matrix metalloproteinase-9, a down-regulator of BBB function, from brain pericytes. Furthermore, CsA-treated pericytes showed elevated expressions of the inflammatory mediators, IL-6 and MCP-1. These findings suggested that brain pericytes may be a key player in the occurrence of CsA-induced neuronal adverse effects.

3-P-219

PKC domains involved in propofol-induced PKC translocation

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Protein kinase C (PKC) alters its localization upon various stimuli and exerts its function at the localized sites, which is called PKC translocation. We found that propofol, an intravenous anesthetic, induced PKC translocation and activated PKC. However, it is not clear which domains of PKC are important for propofol-induced PKC translocation. In this study, we generated mutants lacking each domain of PKC to elucidate the mechanism of propofol-induced PKC translocation and identified the PKC domains involved in this phenomenon.

We used PKC α of conventional PKC and PKC δ of novel PKC. The regulatory region of PKC α consists of C1A and C1B lipid-binding domains and a calcium-binding C2 domain, while PKC δ consists of C1A and C1B domains. GFP-tagged Wild-type (WT) or domain-deficient mutants PKC were expressed in HeLa cells and HUVECs.

WT PKC α was persistently translocated to the plasma membrane (PM) by propofol; deletion of either C1A or C1B or both reduced the duration time of PM translocation. Deletion of all C1A, C1B, and C2 domains abolished translocation to the PM, and PKC was translocated into nucleus. These results suggest that C1A, C1B, and C2 domains are involved in propofol-induced PKC α translocation. WT PKC δ was once accumulated to the Golgi apparatus and then translocated to the PM. Deletion of C1B domain abolished PKC δ translocation to the Golgi and PM, suggesting the involvement of the C1B domain in the propofol-induced translocation of PKC δ. These results reveal that the domains involved in propofol-induced PKC translocation are subtype-specific.

3-P-220

Spatiotemporal analysis of propofol-induced PKC translocation -Subcellular Localization of PKC Activation by propofol-

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Protein kinase C (PKC) changes its localization upon various stimuli and exerts its function at the localized sites, which is called PKC translocation. We have previously shown that propofol, an intravenous anesthetic, induces PKC translocation and activates PKC. In this study, we attempted to further analyze propofol-induced PKC translocation and visualize PKC activation sites in cells to elucidate the mechanism of propofol-induced PKC translocation.

We selected PKC α of conventional PKC, PKC δ of novel PKC, and PKC ζ of atypical PKC. We transiently expressed GFP-tagged PKCs in HeLa cells and HUVEC. Propofol-induced PKC translocation was observed by time-lapse imaging. Local intracellular PKC activation was analyzed in HeLa cells expressing C kinase activity reporter (CKAR) based on the temporal changes of FRET ratio.

Administration of propofol at 100 μM or higher induced translocation of PKC-GFP. PKC α and PKC δ were significantly translocated to the plasma membrane (PM), and PKC δ was also translocated to Golgi apparatus. PKC ζ was translocated into nucleus. Under Ca^{2+} -depleted conditions, the calcium-sensitive PKC α was translocated into the nucleus rather than the PM. These results reveal that propofol-induced translocation is PKC subtypes specific, and has a mechanism for translocation of PKC into nucleus, unlike receptor mediated-translocation. FRET analysis using CKAR showed that PKC was activated at the PM and Golgi. These results suggest that propofol permeates into cells and activates PKC locally, which may be involved in the effects of propofol.

3-P-221

Vonoprazan decreases tight junction-associated proteins at the blood-brain barrier

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Use of proton pump inhibitors (PPIs) is associated with increased risk of dementia and cognitive impairment. Accumulating evidence indicate that the dysfunction of blood-brain barrier (BBB) in hippocampus was observed in the aged people with prior cognitive impairment, suggesting that BBB dysfunction precedes cognitive impairment. The primary responsibility of the BBB is regulating paracellular permeability to blood-borne substances including plasma proteins. Therefore, we hypothesized that PPIs impair brain endothelial barrier formed by tight junction to increase dementia risk. Here, we investigated whether vonoprazan, a potassium-competitive acid blocker, affects the brain endothelial expression of tight junction-associated proteins (claudin-5, occludin and ZO-1) to induce BBB dysfunction. Primary cultures of rat brain endothelial cells (RBECs) were used. RBECs were treated with vonoprazan (1-30 μM) for 24hr. Vonoprazan dose-dependently decreased mRNA and protein expression levels of claudin-5, occludin and ZO-1. mRNA levels of Icam-1 and Vcam-1 were not affected by vonoprazan, suggesting that vonoprazan decreased tight junction in RBECs independently of inflammatory process. These results suggest that vonoprazan could induce BBB dysfunction, leading to the increased risk of dementia.

3-P-222

Decreased brain uptake of cyclosporin A and tacrolimus in the inflammatory bowel disease mice.

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Calcineurin inhibitors, typical immunosuppressive agents, are used for medical treatment of the steroid-refractory ulcerative colitis inflammatory bowel disease (IBD). Increased brain transport of the calcineurin inhibitor cyclosporin A elevates the risk for the occurrence of neurotoxic adverse effects including tremors under the pathological conditions such as partial hepatectomy. However, it has not been well known whether IBD regulates the blood-brain barrier (BBB) permeability for calcineurin inhibitors. Here, we used C57BL/6 mice treated with 3.5 % sodium dextran sulfate (DSS) for 12 days as an IBD model to evaluate brain uptake of the cyclosporin A and tacrolimus using an in situ transcardial perfusion technique. [^{3}H]-Cyclosporin A and [^{3}H]-tacrolimus uptake in the brain of DSS mice were significantly decreased. The increased expression levels of the P-glycoprotein were observed in brain microvasculatures of the DSS mice, suggesting that P-glycoprotein functions may be up-regulated in DSS mice. These findings demonstrate that IBD model mice exhibit decreased transport of the calcineurin inhibitors across BBB by up-regulating P -glycoprotein activity. Thus, IBD may not be included in the risk factors for the neurotoxic adverse effects of calcineurin inhibitors.

3-P-223

Maternal obesity disturbs nurturing behaviors in mice

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Maternal obesity is reported to affect the offspring outcomes, such as increasing the risk of psychiatric disorders and metabolic diseases. In this study, we investigated whether maternal obesity affects nurturing behavior in mice. Eight-week-old female C57BL/6J mice were fed with a high-fat diet (HFD) or a regular diet (RD) for 28 days, and then mated with eight-week-old male C57BL/6J mice and continued to be fed HFD. There was no significant difference in pregnancy rate and number of pups between the mice fed with HFD and the mice fed with RD. In contrast, the mice fed with HFD tended to abandon the offspring and the survival rate of offspring born from the mice fed with HFD was significantly lower than that of offspring born from the mice fed with RD. These results suggest that HFD intake impairs psychological functions in female mice. Thus, we examined whether mice fed with HFD for 28 days show impairment of social behavior using social interaction test. The body weight of the mice fed with HFD was significantly increased compared to that of the mice fed with RD. In the three-chambered social interaction test, the social novelty preference of the mice fed with HFD was impaired. Taken together, our results suggest that HFD intake impairs social behavior in female mice, which disturb nurturing behavior.

3-P-224

Involvement of Peripheral PACAP-PAC1 Receptor Signaling System to Itch Sensation in Atopic dermatitis mice

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Previously, we demonstrated that both intradermal (i.d.) and intrathecal (i.t.) injection of PACAP (1pmol-1nmol) elicited scratching/biting behaviors in mice, and they are suppressed by pretreatment with μ-opioid receptor antagonist naltrexone. Furthermore, we showed that i.t. and i.d. PACAP-elicited scratching/biting behaviors are inhibited by co- injection of small-molecule PAC1 receptor antagonist PA-8 (1 nmol). These results suggests that both peripheral and spinal PACAP/PAC1 receptor signaling systems are involved in the transmission of itch sensations. However, it is not yet clear that PACAP/PAC1 receptor signaling systems are involved in what kind of pruritus. In this study, the importance of PACAP/PAC1 receptor signaling systems were evaluated using dinitrofluorobenzene (DNFB)-induced atopic dermatitis model mice. Repetitive application (once a week) of DNFB (1.5%) to the skin of mice elicited itch- like behaviors. Single oral administration of PA-8 (30 mg/kg) significantly suppressed DNFB-induced itch-like behaviors, suggesting the involvement of PAC1 receptors in atopic dermatitis. Application of PA-8 (0.5% in solvent (DMSO : 100% ethanol = 1 : 9)) to the skin also significantly suppressed the DNFB-induced itch-like behaviors. The result suggests that PACAP/PAC1 receptor signaling systems in the skin are involved at least in part in the itch of atopic dermatitis. Blocking peripheral PAC1 receptors may be one of the new strategies for managing itch sensations of atopic dermatitis.

3-P-225

Analgesic effect of topical application of TRP antagonists to gingiva on orthodontic force-induced pain in rats

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The electrical stimulation to gingiva induced the jaw-opening reflex (JOR). The threshold for inducing JOR (TH) is significantly reduced by application of orthodontic force to the tooth of the stimulating region. The intraperitoneal administration of TRPV1 antagonist significantly inhibited orthodontic force-induced excitation of JOR. However, the existence of adverse side effects (e.g., hyperthermia) of general administration of TRPV1 antagonists provides the necessity to investigate improved strategies. In this study, TRPV1 (AMG9810) and or TRPA1 (A-967079) antagonists were topically applied to gingiva, and the orthodontic force-induced JOR excitation was investigated with other features (e.g., inflammatory cytokines, and rectal and gingival temperatures). TRP antagonist(s) were applied to cervical gingiva immediately after (D0) or one day after (D1) orthodontic force application, and JOR TH was examined on D1. TRP antagonists significantly and dose-dependently increased JOR TH next day, in both D0 and D1 when applied alone. Furthermore, cocktail application of TRPV1 and TRPA1 antagonists (4% each) showed a cooperative/synergistic analgesic effect in both D0 and D1. In D0, the cooperative analgesic effect was associated with a significant reduction of CINC-2 in periodontium (Western blotting assay). In addition, rectal or gingival temperatures did not alter across the experiment. Taken together, topical application of TRP antagonists cocktail may reduce clinical orthodontic pain via CINC-2 reduction without adverse side effects.

3-P-226

Mechanisms of early afterdepolarization-mediated lethal arrhythmias in patients with long QT syndrome type II: an *in silico* study

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Reentry is a basic mechanism of cardiac arrhythmias. In particular, torsades de pointes (TdP) is a spiral excitation wave reentry that meanders through the ventricles. Based on clinical studies showing that early afterdepolarization (EAD) precedes the development of TdP in patients with long QT syndrome type II, it has been suggested that ventricular arrhythmias are triggered by the development of EAD mediated by excessive prolongation of the action potential of ventricular myocytes inducing QT prolongation in ECG. However, the role of EAD in the generation of TdP remains unclear. In the present study, we investigated the relationship between EAD and TdP initiation by constructing a 6 × 6 cm (600 × 600 units) myocardial sheet model consisting of a human ventricular myocyte model (Kurata et al., Biophys J, 2005) and performing simulations of excitation propagation. The EADs were assumed to occur in islands (clusters) in the ventricular tissue, and the relationship between the change in the number of EAD clusters and the spiral excitation wave (TdP) initiation was investigated. In the case of a single island (400 x 400 unit EAD cluster), spiral excitation wave initiations did not occur. On the other hand, spiral excitation waves were caused by dividing the 400 × 400 unit EAD cluster into 4, 9, and 16 islands. However, no spiral excitation occurred in the 25 clusters. This suggests that not only the spatial distribution of EAD clusters but also the cluster size influences the TdP initiation. Clustering EAD-evoked cardiomyocytes and discontinuous distributions of the clusters may promote the TdP initiation, i.e., may increase the risk for lethal arrhythmia in patients with LQT2.

3-P-227

hERG channel inactivation and drug binding

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The voltage-gated potassium channel, KV11.1, encoded by the human ether-a-go-go related gene (hERG) is expressed in cardiac myocytes, where it is crucial for the membrane repolarization of the action potential. hERG is implicated in a number of drug-induced arrhythmias, caused by long QT syndrome. Gating of hERG is characterized by rapid, voltage-dependent, C-type inactivation, which blocks ion conduction and is suggested to involve constriction of the selectivity filter. To explore conformational changes associated with hERG inactivation, we use RosettaRelax to simulate the effects of hERG mutations. We show that a lateral shift of residue F627 in the selectivity filter into the central channel axis along the ion conduction pathway. Non-inactivating mutations S620T and S641T showed a potential blocking mechanism of F627 rearrangement, preventing it from shifting into the conduction pathway during the proposed inactivation process. Furthermore, drug docking results correlate well with existing experimental evidence of protein-ligand contacts between high-affinity hERG blockers and key residues Y652 and F656 inside the pore cavity, in addition to illuminating potentially new ligand binding interactions in the inactivated state fenestration region.

3-P-228

Evaluation of RyR2 inhibitors as anti-arrhythmic drugs using novel CPVT model mice

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Mutations in type 2 ryanodine receptor (RyR2), a Ca^{2+} release channel on the sarcoplasmic reticulum, are known to cause ventricular arrhythmias such as catecholaminergic ventricular tachycardia (CPVT). Because conventional anti-arrhythmic drugs sometimes fail to stop arrhythmias in CPVT patients, development of new antiarrhythmic agents that directly target RyR2 is desired. Recently we have screened a well-characterized chemical compound library and found 2 compounds with RyR2 inhibitory action, riluzole and chloroxylenol. In this study, we aimed to verify the anti-arrhythmic effects of these RyR2 inhibitors using a novel CPVT mouse model (RyR2-I4093V) which was established by screening of an ethylnitrosourea-induced mutagenized mice library. ECG records were obtained from the I4093V mice under anesthesia and drugs were intraperitoneally administered. The I4093V mice showed little arrhythmias at two months of age, but exhibited severe ventricular arrhythmias by β-agonist administration. Moreover, frequent spontaneous arrhythmias were observed without β-stimulation at 4-5 months old. Conventional anti-arrhythmic agents (flecainide, pilsicainide, atenolol) and the two novel RyR2 inhibitors suppressed the spontaneous arrhythmia to varying degrees. Our results indicate that the I4093V mouse is a useful model for evaluation of antiarrhythmic drugs and that RyR2 inhibitors are promising candidates for novel anti-arrhythmic drugs.

3-P-229

Enhanced susceptibility to catecholamine-induced arrhythmias in CNNM2-deficient mice

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Magnesium ion (Mg^{2+}) is an important divalent cation and plays an essential role in various cellular functions. Therefore, cellular Mg^{2+} concentration is tightly regulated by multiple Mg^{2+} channels/transporters. Mg^{2+} deficiency or abnormal Mg^{2+} metabolism is known to lead to various cardiovascular diseases. Cyclin M2 (CNNM2) is one of the members of Na^{+}/Mg^{2+} exchangers, participating in Mg^{2+} reabsorption in kidney tubules. However, the pathophysiological significance of CNNM2 in cardiovascular diseases remains unclear. In this study, we generated CNNM2-deficient mice using CRISPR/Cas9 system. CNNM2-deficient mice, fed with a normal Mg^{2+} diet, exhibited significantly decreased serum Mg^{2+} levels and increased urinary Mg^{2+} excretion compared to wild-type mice. In addition, the systolic blood pressure in CNNM2-deficinet mice was lower than that in wild-type mice. Cardiac functions in CNNM2-deficient mice were normal, but interestingly, the susceptibility to epinephrine/caffeine-induced arrhythmia was enhanced. The enhanced susceptibility to epinephrine/caffeine-induced arrhythmia in CNNM2-defocient mice was alleviated by the treatment with a high Mg^{2+} diet for 4 weeks. These results suggest that CNNM2-deficiency with hypomagnesemia is linked to catecholamine-induced arrhythmias.

3-P-230

Protective effect of *N*-acetylcysteine against the cytoskeletal injury induced by cigarette tobacco extract and heated tobacco products in C2C12 myoblasts

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Sarcopenia is an age-related skeletal muscle disorder characterized by the progressive loss of muscle mass and function, resulting in physical disability and mortality. Aberrant reactive oxygen species (ROS) generation and oxidative tissue damages in skeletal muscles have been implicated as one of key causative factors in the multiple mechanisms of sarcopenia. Tobacco smoke contains many toxic chemicals including highly unstable free radicals and ROS. Currently, heated tobacco products (HTPs) are widely used as substitutes for cigarette smoking around the world. However, it is poorly understood the effects of these new generation tobacco products on skeletal muscles. In the present study, we investigated the muscular injury induced by cigarette smoke extract (CSE) and HTPs using murine myoblast C2C12 cells as an *in-vitro* model. The exposure of either CSE or HTPs for 24 h decreased the cell viability in C2C12 myoblasts, which protected by treatment with 1 mM *N*-acetylcysteine (NAC). In addition, the differentiation to myotube and the expression of myogenic marker protein were suppressed by CSE or HTPs exposure. Interestingly, the formation of stress fibers was disrupted by the treatment with CSE, and the aberrant membrane protrusion was observed. These abnormalities in cytoskeletons and cell shapes were also rescued by the treatment of NAC. These findings suggested that CSE and/or HTPs can damage skeletal muscles through the impairment of cytoskeleton formation, which may contribute to the progress of muscular damages, and age-related muscular diseases.

3-P-231

3-P-232

Relationship between synchronized tissue-wide Ca^{2+}oscillations and blood flow fluctuations induced by acetylcholine in rat submandibular gland

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Intravenous infusion of acetylcholine (ACh) caused Ca^{2+}oscillations synchronized at the tissue level in rat submandibular gland (SMG). To examine the mechanism of the synchronized Ca^{2+}oscillations in SMG, we monitored Ca^{2+}responses and blood flows (BF) in live animals using the intravital Ca^{2+}imaging system and the laser speckle BF imager. Monitoring of BF dynamics demonstrated that ACh caused oscillatory changes in BF in SMG. The oscillatory changes in BF showed a diverse spacial patterns occurring from anterior, intermediate, lateral, and posterior regions of SMG. Preadministration of calciumantagonist, nicardipine, an angiotensin II (Ang II) receptor blocker, irbesartan, or an angiotensin converting enzyme inhibitor, enalapril (Ena), changed the BF oscillations to a sustained increase in BF without fluctuation. After the attenuation of BF fluctuation by Ena, intravenous infusion of Ang II recovered BF oscillation. Theseresults indicate that vasoconstriction by Ang II plays a critical role in the regulation of BF oscillations. In addition, BF oscillation do not require fluctuations ACh and Ang II concentrations in the plasma. Furthermore, simultaneous monitoring of $Ca^{2+}$and BF dynamics in SMG revealed that the increase in intracellular Ca^{2+}concentration preceded the increase in BF in most cases (85%, 71/84 oscillations, in 4 rats). These results suggest that Ca^{2+}response of acinar cells leads vasodilation through the production of localized diffusible substances in the SMG.

3-P-233

Centrally administered corticotropin-releasing factor facilitates the rat micturition reflex via brain glutamatergic receptors

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Roles of brain corticotropin-releasing factor (CRF), a representative stress-related neuropeptide, on micturition reflex are controversial, both suppressive and facilitatory roles are reported. In this study, we examined effects of intracerebroventricularly (icv) administered CRF on micturition reflex in urethane-anesthetized (0.8 g/kg, ip) male Wistar rats (300-450 g). A catheter was inserted into the bladder to perform cystometry. Three hours after the surgery, CRF (1 or 3 nmol/rat) was icv administered. Effects of icv pretreated CP154526 (CP, CRF1 receptor antagonist, 30 or 100 nmol/rat), K41498 (K, CRF2 receptor antagonist, 30 nmol/rat), MK-801 (MK, NMDA receptor antagonist, 3 or 10 nmol/rat) or DNQX (AMPA receptor antagonist, 1 or 3 nmol/rat) on the CRF-induced responses were also examined. CRF dose-dependently shortened intercontraction intervals (ICI), an index of micturition frequency. The CRF-induced ICI shortening was significantly attenuated by icv pretreated CP, MK or DNQX, respectively. On the other hand, K showed no significant effect on the CRF-induced response. These results suggest that brain CRF can facilitate the micturition reflex via brain NMDA/AMPA glutamatergic receptors in rats.

3-P-234

Effects of temozolomide and PCPA on micturition function in mice with an enriched environment

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We recently reported that enriched environment (EE) was significantly decreased voiding frequency and total voided volume in BALB/c female mice. However, the mechanism is unknown. In this study, we investigated whether temozolomide, a neurogenesis inhibitor, and PCPA (*p*-chlorophenylalanine), a serotonin synthetic enzyme inhibitor, were affected micturition function in mice with EE. Temozolomide was injected in BALB/c female mice on 3 days every week for a total of 6 weeks. The mice were reared in the EE or standard environment (SE) from the day after the end of temozolomide administration. PCPA (100 mg/kg, i.p., administered for 4 days) was injected in mice between 22 and 23 weeks from the start of EE. Micturition activity of freely moving mice was measured the day after the last day of PCPA administration. In the light period, EE was significantly decreased in voiding frequency and total voided volume compared to SE. Temozolomide was significantly increased in voiding frequency and total voided volume in the EE group at the light period. In the dark period, PCPA was significantly decreased in total voided volume, mean voided volume, voiding duration, and maximum flow rate, regardless of the rearing environment. These results suggested that neurogenesis may be related to the enrichment effects on micturition function.

3-P-235

Effects of reactive oxygen species on hepatic indoxyl sulfate production

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We reported that a super oxide dismutase mimetic agent, tempol, inhibited elevation of indoxyl sulfate (IS) and recovered impaired vascular function in adenine-induced kidney disease rats. These results suggested that reactive oxygen species (ROS) accelerated IS production or tempol directly inhibited IS production. The aim of study is to clarify the effect of tempol on IS production. We measured IS production by the use of rat liver S9 fraction *in vitro*. Tempol weakly inhibited hepatic IS production (IC_{50} of 138 mM). We examined the effect of ROS on IS production by using H_2O_2 or free radical generators, 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH). Although H_2O_2 failed to affect IS production, AAPH accelerated IS production. These results suggested that ROS accelerated IS production. In conclusion, tempol seems to inhibit IS production through both directly inhibit sulfotransferase or cytochrome P450 and reduction of ROS. This finding suggests that antioxidant may be useful therapy of cardiorenal syndrome mediated by uremic toxin.

3-P-236

Involvement of Indoxyl Sulfate in Vascular Endothelial Dysfunction associated with Ischemic Acute Kidney Injury

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In our previous study, we found that impaired renal function due to ischemic acute kidney injury (IAKI) recovered over time; however, the endothelial function of the thoracic aorta deteriorated from the time when renal function shifted toward recovery. In the present study, we investigated the influence of indoxyl sulfate (IS) on vascular endothelial dysfunction after the onset of IAKI by administering AST-120, which can remove IS. IAKI was induced by occlusion of the left renal artery and vein for 45 min, followed by reperfusion 2 weeks after contralateral nephrectomy. AST-120 (2.5 g/kg, p.o.) was administered at 3, 6, and 24 hours after reperfusion. At 1 and 7 days after reperfusion, a significant increase in blood IS levels was observed along with worsening of renal function. On the other hand, AST -120 administration significantly suppressed the blood IS levels but had no effect on the decline in renal function after reperfusion. Furthermore, renal function and blood IS levels at 28 days after reperfusion were not significantly different among all groups. In contrast, vascular endothelial function was markedly attenuated 28 days after reperfusion in the IAKI model. In addition, administration of AST-120 did not clearly improve this impaired vascular endothelial function, suggesting that IS may not be involved in the vascular endothelial dysfunction that occurs after the onset of IAKI. Therefore, further investigation of the detailed mechanism of the vascular endothelial dysfunction that occurs after the onset of IAKI is considered necessary.

3-P-237

Evaluation of urinary biomarkers in ischemia/reperfusion-induced renal injury

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Acute kidney injury (AKI) has been linked to an increased risk of developing chronic kidney disease (CKD). In the pathophysiology of AKI, oxidative stress plays a pivotal role. Previously, we reported that vanin-1, which is involved in oxidative stress, is associated with renal tubular injury. This study aims to determine whether urinary vanin-1 is a biomarker for early diagnosis of AKI in two experimental models: in vivo and in vitro. In a rat model of AKI induced by ischemia/reperfusion (I/R), ischemic AKI was induced in uninephrectomized rats by clamping the left renal artery for 45 min and then reperfusing the kidney. After 1 day of the treatment, urinary N-acetyl-β-D-glucosaminidase (NAG) exhibited a significant increase, but decreased on Day 2 in I/R rats. Serum creatinine (SCr) in I/R rats showed higher than sham-operated rats, but did not reach significance. In contrast, urinary vanin-1 significantly increased on Day 1 and remained significant high level on Day 2 in IR rats. Renal vanin-1 protein was decreased on Days 1 and 3. In line with these findings, immunofluorescence staining demonstrated that vanin-1 was attenuated in the renal proximal tubules in I/R rats. In vitro, the supernatant from HK-2 cells under hypoxia/reperfusion significantly highly included vanin-1 as well as KIM-1 and NGAL. In conclusion, our results suggest that urinary vanin-1 might be a potential novel biomarker for AKI induced by I/R.

3-P-238

Evaluation of micturition function of water load model using cynomolgus monkey

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Backgrounds: Although there are reports of cystometry and water load models in rodents, there are few reports on the evaluation of micturition function in cynomolgus monkeys. This time, we evaluated the micturition function of a water load model using cynomolgus monkeys. As a positive control, desmopressin acetate used for nocturia was used.
Methods:Water loading was performed after administration of desmopressin acetate or vehicle, and micturition volume was continuously measured up to 6 hours after water loading. From the obtained measurement data, the total amount of urination volume, the number of urination, and the amount of urination volume once were calculated.
Conclusion and Discussion: In a water loading model of cynomolgus monkeys treated with desmopressin acetate, urine output decreased with increasing dose. As a result, it was suggested that the water load model using cynomolgus monkeys can evaluate the micturition function.

3-P-239

Interaction of AGN-1, which binds to protein phosphatase 6, with tau alternative splicing variants mRNAs in neuroblastoma cells

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We cloned AGN-1, which interacted with protein phosphatase 6, as a molecule highly expressed in nephritic rat kidney. Our previous studies revealed that in neuronal cells (Neuro2a cells), AGN-1 was co-localized with U1-70K, a constitutive protein of U1snRNP, and splicing factor SC35 which participates in the synthesis of tau alternative splicing variants, 4R-tau and 3R-tau. Moreover, our study showed that AGN-1 knock-down decreased mRNA expression ratio of 4R-tau to 3R-tau in Neuro2a cells. In this study, to clarify the mechanism underlying the change in the expression pattern of the tau alternative splicing variants, we investigated whether AGN-1 binds to 4R-tau and 3R-tau mRNAs. RNA immunoprecipitation assay using anti-AGN-1 antibody and cytosol fraction of Neuro2a cells resulted in PCR fragments derived from 4R-tau and 3R-tau mRNAs. *In vitro* synthesized 4R-tau and 3R-tau mRNAs bound to AGN-1 protein prepared by immunoprecipitation, and the binding affinity of 3R-tau mRNA seemed to be higher than that of 4R-tau mRNA. Additionally, AGN-1 was co-immunoprecipitated with U1snRNP constitutive proteins. These results suggest that AGN-1 protein interacts with 4R-tau and 3R-tau mRNAs, and U1snRNP constitutive proteins including AGN-1 may involve in the interaction.

3-P-240

A candidate molecule for non-invasive biomarkers in renal Nrf2 activation

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Background

NF-E2-related factor 2 (Nrf2), a master regulator of the oxidative stress response, plays an important role in influencing the severity of the renal injury. However, non-invasive biomarkers to detect the Nrf2 activation in the kidney have yet to be elucidated. We focused on urine exosomes as a source of non-invasive biomarkers. Exosomes are released from all renal epithelial cells, and contain nucleic acids, proteins, and lipids. In this study, in order to search biomarkers for the Nrf2 activation, we employed bardoxolone methyl (BARD), a Nrf2 activator, and analyzed RNA in urine exosomes.

Methods

Male Sprague-Dawley rats were divided into two groups: the vehicle and BARD groups (10 mg/kg). Urine samples were collected for 6 hours after BARD administration, and exosomes were isolated from the urine using the polymer precipitation method. The kidneys were collected at 6 hours after the dosing. RNAs were extracted from urine exosomes and kidneys, and examined by microarray analysis. The urine exosomes were also analyzed by next generation sequencing.

Results

Among the RNAs in the kidney and urine exosomes, the expressions of eight mRNAs and one miRNA were commonly altered after the treatment with BARD. Next-generation sequencing showed that BARD altered only the expression level of pirin mRNA among RNAs mentioned above.

Conclusion

These results suggest that pirin mRNA in urine exosomes might be a useful biomarker to detect the renal activation of Nrf2.

3-P-241

Downregulation of progesterone receptor membrane component 1 (PGRMC1) enhances cyclooxygenase 2 expression via upregulating FOXO1 expression in the process of human endometrial stromal cells differentiation

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Decidualization is a differentiation process of the endometrial stromal cells (ESCs) in preparation for embryo implantation. ESCs differentiate into decidual cells by the action of progesterone (P4) and the mediator cAMP, and produce specific markers such as IGF-binding protein 1 and prolactin during the mid-secretory phase of the menstrual cycle.

Decidualized cells express FOXO1, a transcription factor of the decidual markers and cyclooxygenase 2 (COX2) which is involved in the production of prostaglandins.

We have previously showed that P4 receptor membrane component 1 (PGRMC1), a non-canonical P4 receptor, is decreased in secretory phase of endometrium, and inhibition of PGRMC1 function accelerates ESCs decidualization. In this study, we explored the roles of PGRMC1 in the regulation of FOXO1 and COX2 expression in ESCs. Primary human ESCs were pretreated with the inhibitor of PGRMC1 (AG205) or PGRMC1 specific siRNA and then stimulated with P4 and dibutyryl-cAMP (P4/db-cAMP) to induce differentiation. In the presence of P4/db-cAMP, either the inhibitor or knockdown of PGRMC1 significantly enhanced FOXO1 and COX2 expression. Notably, silencing FOXO1 expression repressed the P4/db-cAMP-induced COX2 expression. In addition, the inhibitor of NF-kB signaling pathway suppressed the COX2 expression in PGRMC1-downregulating ESCs. Our results suggest that downregulation of PGRMC1 in the secretory phase of endometrium may promote decidualization via induction of FOXO1 and COX2 expression for the establishment of pregnancy.

3-P-242

The number of litters in V1aR-KO mice decreased due to the abnormal growth of the fetus in utero

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Vasopressin (VP), which is one of the neurohypophyseal hormone, can play diverse roles via specific receptors belonging to a family of GPCRs. There are three subtypes of VP receptors–V1aR, V1bR, and V2R. Previous our studies reported that V1aR-KO female mice showed various reproductive abnormalities. In this analysis, we focused on the mechanism of the decrease of litter size and tried to identify the period when the fetuses drop out.
First, we performed the staining of the implantation site (IS) at the day 5 of pregnancy (E5). However, the number of IS were not different. This observation suggests that the decrease of litter size observed in the V1aR-KO mice occurred after implantation. We next measured the litter size and the weights of fetuses and placentas at E14, E18, and E19. As a result, the weights of fetuses and placentas were smaller in the V1aR-KO mice compared with the wild-type. Intriguingly, aberrant fetuses are frequently observed at E14, E18, and E19 in the V1aR-KO mice and the litter sizes of the normal fetuses were smaller. Especially, omphalocele was observed in 13.5 % of E19 V1aR-KO fetuses. In general, fetuses with omphalocele die after birth. Therefore, the significant decrease in the number of litters observed in V1aR-KO mice may be due to the abnormal growth of the fetus in utero, such as omphalocele.

3-P-243

Intracochlear inflammation reduces the number of synapses between inner hair cells and the cochlear nerve

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Sensorineural hearing loss (SNHL) causes irreversible hearing impairment that is in many cases and thus significantly reduces the quality of life. Our previous studies have reported that repeated exposure to moderate noise reduces the number of synapses between inner hair cells (IHC) and the cochlear nerve (IHC synapses). This study aimed to clarify the cause of this decrease in the number of synapses due to repeated noise exposure. IHC synapses were visualized by immunostaining with specific antibodies against c-terminal binding protein 2 (CtBP2) and glutamate receptor subunit A2 (GluA2). Repeated noise exposure increased the expression of the interleukin-1β gene (*Il1b*) and cyclooxygenase (COX)-2 protein in the IHC, thereby causing intracochlear inflammation. Treatment using continuous PLX3397 (290 ppm in MediDrop sucralose, a colony-stimulating factor-1 receptor inhibitor) for 1 week decreased the number of macrophages in the cochlea and, significantly, the number of IHC synapses due to repeated noise exposure. NS-398 (5 mg/kg, COX-2 inhibitor) significantly suppressed the loss of IHC synapses caused by repeated noise exposure. In addition, intraperitoneal administration of lipopolysaccharide (1 mg/kg) increased cochlear *Il1b* at 4 h post-dose and significantly decreased the number of IHC synapses at 24 h post-dose. These data suggest that intracochlear inflammation is involved in the reduction of the number of IHC synapses.

3-P-244

Gene expression profiles in lacrimal glands after retinal damage of mice

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Early detection of such retinal diseases as glaucoma and age-related macular degeneration (AMD) is important to prevent blindness. There have been reports of changes in some components in the tears of glaucoma and AMD patients, suggesting tears' potential usefulness in screening for retinal diseases. We hypothesized that retinal damage might alter gene expression in the lacrimal gland. We caused retinal damage in mice by intravitreal injection of *N*-methyl-D-aspartate (NMDA) or excessive light exposure. Hematoxylin and eosin staining showed no histological changes in the lacrimal glands of animals whose retinas had been damaged. However, RNA sequencing of lacrimal glands on the 3rd day after the retinal damage showed that several hundreds of gene expressions have altered in both the NMDA and light group. Further gene set enrichment analysis indicated that both types of retinal damage activated the immune system in the lacrimal glands. This study shows the first demonstration that retinal damage can alter gene expression in the lacrimal glands, and it might lead to a novel non-invasive screening method for retinal diseases.

3-P-245

Rapamycin attenuates noise-induced hearing loss by promoting autophagy in the cochlear lateral wall

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Autophagy, a self-digestion intracellular catabolic process, plays a crucial role in cellular homeostasis. Our previous studies demonstrated that noise at 110-dB sound pressure level (SPL) produced permanent hearing impairment. In the present study, we prepared an animal model of permanent hearing loss and sought to determine whether rapamycin (autophagy activator) have preventive effect on noise-induced hearing loss in the model mice. To determine the activation of autophagy following noise exposure, we evaluated the expression of LC3-II (LC3 = LC3-II/LC3-I, autophagy marker) in the cochlea after a 1-h exposure to noise at 110 dB SPL. Immunoblot analysis revealed that noise exposure produced a dramatic increase in the LC3-II level of the cochlea at 1-h post-exposure. Moreover, rapamycin significantly alleviated hearing impairment induced by exposure to noise at 110-dB SPL. In addition, immunohistochemistry analysis revealed that rapamycin was effective in enhancing expression of LC3 in the cochlear lateral wall. Taken together, our data suggest that autophagy activator is a candidate of preventive drugs for sensorineural hearing loss.

3-P-246

Valproic acid accelerates the developmental cell death and delays the synaptogenesis in the mouse retina

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Valproic acid is widely used as an antiepileptic drug. However, valproic acid use during pregnancy increases the risk of neurodevelopmental disorders in the offspring. In this study, we examined the effects of valproic acid on retinal development in neonatal mice. Mice were subcutaneously injected with valproic acid or vehicle once a day from postnatal day (P) 0 to P7. The mice were sacrificed on P4, P8, P14, and P21. Until P8, the cell number in the ganglion cell layer and the thickness of retinal layer decreased in mice treated with valproic acid. On P8, the outer plexiform layer was evident in vehicle-treated mice but was not apparent in valproic acid-treated mice. The numbers of bipolar and horizontal cells in valproic acid-treated mice were significantly less than those in vehicle-treated mice. The appearance of immunoreactivity for synaptophysin (a presynaptic marker) in the outer and inner plexiform layers was delayed in valproic acid-treated mice. These results suggest that exposure to valproic acid during retinal development leads to the acceleration of developmental cell death and the delay in synaptogenesis in the retina. Therefore, when neurologists prescribe valproic acid for epileptic pregnant women, they should pay special attention to the increased risk of inducing visual dysfunction in their babies.

3-P-247

Endogenous apelin is protective against retinal dysfunction in type 1 diabetic Akita mice

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Retinal neurodegeneration occurs in the early stages of diabetic retinopathy and contributes to the development of vascular lesions, which lead to macular edema and traction retinal detachment. Apelin is an endogenous peptide ligand of the G protein-coupled receptor APJ, and the apelin/APJ system has recently been reported to play a crucial role in maintenance of homeostasis in various tissues, including retina. In the present study, we investigated the protective effect of endogenous apelin against retinal dysfunction in type 1 diabetic Akita mice. Retinal function was evaluated by electroretinography. mRNA expression levels were measured by real-time RT-PCR. Electroretinogram responses in Akita mice were declined with age and significantly decreased at 4 months of age. Apelin and APJ mRNA expression levels in the retina of 4-months-old Akita mice were lower than those in the age-matched wild type mice. The electroretinogram reductions in apelin deficient Akita mice were marked compared with Akita mice. Apelin deficiency did not affect transitions in the blood glucose levels in Akita mice. These results indicate that endogenous apelin is protective against retinal dysfunction in Akita mice, suggesting that apelin prevents retinal neurodegeneration in the early diabetic retinopathy.

3-P-164

Suppression of M1 macrophage activation and osteoclast differentiation by Apocynaceae plant extract

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Macrophages play important roles in immune response and tissue homeostasis. In response to various extracellular stimuli, macrophages polarize and differentiate into subgroups such as M1 and M2 macrophages, and osteoclasts. Excessive M1 macrophage and osteoclast activation is associated with inflammatory diseases including rheumatoid arthritis. Here, we find the Apocynaceae plant (AP) extract suppresses LPS-induced M1 activation and RANKL-induced osteoclast differentiation in mouse bone marrow-derived macrophages. Expression levels of LPS-induced nitric oxide and pro-inflammatory cytokines such as TNF, IL-1β and IL-6 and RANKL-induced osteoclast marker genes such as NFATc1, CTSK, ACP5, DC-STAMP, ATP6V0D2 were suppressed by AP extract. AP extract inhibited LPS- and RANKL-induced phosphorylation of Akt and IκBα degradation. Furthermore, AP extract significantly reduced PDK1 kinase activity. Suppression of osteoclast differentiation by AP extract was partially rescued by a PDK1 activator PS-48. These results suggest that the inhibitory effects of AP extract on LPS-induced M1 macrophage polarization and RANKL-induced osteoclast differentiation are associated with suppression of PDK1-Akt-NF-κB signaling pathway and may provide an important information for developing new therapeutic agents for inflammatory diseases.

3-P-248

Synthetic retinoid bexarotene inhibits differentiation of inflammatory osteoclasts

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Macrophages differentiate into osteoclasts upon stimulation by RANKL. In inflammatory lesions such as rheumatoid arthritis, direct stimulation of macrophages by inflammatory cytokines promotes osteoclast differentiation, which is speculated to contribute to pathological bone destruction. Therefore, inhibition of inflammatory osteoclast differentiation may ameliorate bone destruction. All-trans retinoic acid (ATRA), the active form of vitamin A, activates retinoic acid receptor (RAR), resulting in potent inhibition of RANKL-induced osteoclast differentiation. ATRA also activates retinoid X receptor (RXR). The importance of RXR in inhibiting osteoclast differentiation is unknown. Bexarotene, a synthetic retinoid that exhibits anti-tumor activity, is an agonist specific for RXR. To clarify the effect of RXR-stimulation on osteoclast differentiation, we compared the effects of ATRA and bexarotene on differentiation of RANKL-induced or inflammatory (TNF/IL-6-induced) osteoclasts. Bexarotene inhibited their differentiation by suppressing the expression of Nfatc1, a master regulator of osteoclast differentiation as well as ATRA, suggesting the importance of RXR in suppressing osteoclast differentiation. Bexarotene may contribute to the treatment of pathological bone destruction in inflammatory lesions as drug repositioning candidates.

3-P-249

Inhibitory effect of spermidine on differentiation of inflammatory osteoclasts

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Osteoclasts are differentiated from macrophages by RANKL. On the other hand, proinflammatory cytokines such as TNF and IL-6 directly stimulate macrophages to differentiate into osteoclasts (inflammatory osteoclasts) in a RANKL-independent manner. It has been speculated that the inflammatory osteoclasts contribute to the pathological bone destruction in chronic inflammatory lesions observed in rheumatoid arthritis. Therefore, reducing inflammatory osteoclasts is expected to contribute to the improvement of pathological bone destruction. In this study, we used TNF/IL-6-induced osteoclasts as a model of inflammatory osteoclasts and found spermidine as a molecule inhibiting differentiation of the inflammatory osteoclasts. Spermidine has anti-oxidant and anti-inflammatory properties and various beneficial effects on aging-associated diseases. However, little is known about the effects of spermidine on the differentiation of inflammatory osteoclasts. We found that spermidine had no effect on macrophage viability and inhibited differentiation more potently for inflammatory osteoclasts than for RANKL-induced osteoclasts. The inhibitory effect was found to be partially due to the antioxidant property of spermidine. Spermidine may be effective in the treatment of pathological bone destruction.

3-P-250

Effect of Novel Women's Preservative Herbal Medicine on locomotive syndrome of OVX mice

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It had been showed that osteoporosis in postmenopausal women bring on locomotive syndrome (locomo) which are restricted in one's ability to walk owing to a motor dysfunction. Besides, menopausal disorders symptoms such as fatigue might cause exercising less and aggravate locomo. In this study, we examined effects of novel women's preservative herbal medicine on symtoms like locomo of ovariectomized (OVX) mice.

The female ICR mice of 10-weeks old were received ovariectomy or sham operation. The mixed diet of herbal medicine (1 % or 2 %) were ingested to each groups for 5 weeks starting from 8 weeks after OVX. The spontaneous activity of the mice was evaluated by using an activity sensor at dark term (19:00-7:00). At the end of study period, animals were autopsied and bone and soleus muscle were collected for micro-CT, bone strength and gene expression study.

In the result, herbal medicine (2%) significantly suppressed decreased the spontaneous activity in dark term of OVX model mice. Additionally, the decrease in soleus muscle weight per body weight and bone strength due to OVX was improved by ingestion of herbal medicine (2 %). Moreover tartrate-resistant acid phosphatase (TRAP) and TNF receptor-associated factor 6 (TRAF6) expression level involved in bone resorption were increased by OVX and significantly recovered by herbal medicine (2 %). The present result suggested that this herbal medicine improved condition of reduced mobility mobility due to impairment of bones and muscles induced OVX.

3-P-251

C-type natriuretic peptide stimulates bone growth by potentiating autonomic Ca^{2+} entry in growth plate chondrocytes

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C-type natriuretic peptide (CNP) is an endocrine factor that potently promotes long bone growth by activating natriuretic peptide receptor 2 (NPR2) which is equipped with guanylate cyclase on the cytoplasmic side. However, its signaling pathway is unclear in growth plate chondrocytes. We have recently developed an original method for live imaging of intracellular Ca^{2+} in growth plate chondrocytes and found a pathway in which spontaneous Ca^{2+} fluctuations mediated by transient receptor potential cation channel subfamily M member 7 (TRPM7) promotes bone growth. In the present study, our Ca^{2+} imaging data indicated that CNP potentiated spontaneous Ca^{2+} fluctuations in growth plate chondrocytes. Further pharmacological analyses suggested that CNP induced hyperpolarization mediated by big-conductance Ca^{2+}-dependent K^{+} (BK) channels, activating TRPM7-mediated Ca^{2+} entry. Indeed, *ex vivo* organ culture analysis indicated that CNP-facilitated bone growth was abolished by chondrocyte-specific *Trpm7* gene ablation. Collectively, we proposed a new CNP signaling axis by which CNP promotes bone growth by activating intracellular Ca^{2+} signaling. These findings might contribute to the development of chemical drugs not only for developmental disorders but also for the artificial modification of body sizes in farm and pet animals.

3-P-252

Osteogenetic effects of KY-273, a diphenylether derivative, in mesenchymal stem cells and ovariectomized rats.

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Orally used bisphosphonates, bone resorption inhibitors, and PTH and anti-sclerostin antibodies for injection, which are both bone formation stimulators, are used for osteoporosis therapy. Oral osteogenetic drugs are desired but have not yet been developed. KY-273 selectively inhibited CDK8/19, whereas siRNA-mediated knockdown of CDK8/19 promoted osteoblast differentiation in mesenchymal stem cells (ST2 cells), suggesting osteogenetic effects of KY-273. In ST2 cells, KY-273 increased the mRNA expression and enzyme activity of ALP, and induced osteoblast mineralization. In ovariectomized (OVX) rats, KY-273 (10 mg/kg/day, p.o.) for 12 weeks gradually increased the cortical bone volume, outer diameter, simulated bone strength and the medullary volume (MV) in femoral epiphysics and diaphysis. The effects of KY-273 were compared with those of alendronate, a bisphpsphonate, and teriparatide, a *parathyroid hormone, in OVX rats.* KY-273 at 3 and 10 mg/kg/day for 8 weeks dose-dependently increased the femur cortical bone volume, MV and simulated bone strength without affecting the trabecular bone volume, accompanied by an increase in blood ALP3, a bone formation marker. Alendronate (3 mg/kg/day, p.o.) markedly increased trabecular bone and slightly increased the cortical bone volume without affecting MV, whereas teriparatide (3 and 10 microgram/kg/day, s.c.) markedly increased both trabecular and cortical bone volumes with reducing MV. The present study revealed that KY-273 has therapeutic effects against osteoporosis via the promotion of bone formation, the characteristics of which are different from those of alendronate or teriparatide.

3-P-253

PARP inhibitor olaparib suppresses osteogenic differentiation in MC3T3-E1 cells.

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Poly(ADP-ribosyl)ation (PARylation) is a reversible post-translational modification catalyzed by the poly (ADP-ribose) polymerase (PARP) family proteins. PARylation regulates many biological processes such as DNA repair, cell death, transcriptional regulation and differentiation. The function of PARP1 has been well-studied in cancer sciences. Recently, a PARP inhibitor, olaparib, was approved as an anti-cancer agent for treatment of breast and ovarian cancer possessing *BRCA1/2* mutations. On the other hand, little is known about the relation of osteogenic differentiation and PARP1. In this study, we analyzed the effects of olaparib on osteogenic differentiation to elucidate the role of PARP1 in the process of osteogenic differentiation.

Pre-osteoblastic MC3T3-E1 cells were induced to differentiate into osteogenic lineage with or without olaparib treatment. Up-regulation of alkaline phosphatase (ALP) activities accompanied with osteogenic differentiation was suppressed by olaparib at an early stage after differentiation induction (at day 14). Furthermore, Alizarin red S staining at 21 days after differentiation induction revealed that calcium deposition was significantly reduced by olaparib treatment. These results suggested that olaparib suppressed osteogenic differentiation of MC3T3-E1 cells in an initial stage of differentiation.

3-P-254

Morphological and functional modification of PC12 cells by bone matrix protein osteocalcin

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Bone matrix protein osteocalcin (OC) was shown to support the development of learning and memory and also prevent anxiety-like behaviors in mice. Although the involvement of GPR158 as the candidate receptor for OC has been reported, the direct effect of OC on the neuronal cells are still unclear. In this study, we investigated both morphological and functional effects of OC on neurons using a model cell line, rat pheochromocytoma cell line PC12 cells.

We detected relatively high expression of GPR158 at both mRNA and protein level, while the expression of the other OC receptor GPRC6A was barely detected in PC12 cells. Treatment of PC12 cells with OC induced transient phosphorylation of ERK, indicated the existence of functional OC receptor in the cells. Proliferation of PC12 cells was promoted in the the presence of 5 to 50 ng/mL of OC. NGF-induced neurite outgrowth was up-regulated by treating the cells with OC. Apoptosis of PC12 cells induced by H_2O_2 was observed as the cleavage of PARP and was suppressed in the presence of OC. The results suggested that OC has direct effect on neuronal morphology and proliferation and also functional cell survival by binding to GPR158 and modulation of downstream intracellular signaling.

3-P-255

Steroidogenesis via multiple purinergic receptors in bovine adrenocortical fasciculata cells.

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[Background] We have previously demonstrated that extracellular ATP induces steroidogenesis via purinergic P2Y receptors (P2YR) in bovine adrenocortical fasciculata cells (BAFC). However, it is not known if extracellular ATP (a very hydrolyzable nucleotide) can survive long enough to act on P2YR during the long incubation period required for BAFC steroidogenesis.
[Purpose] We analyzed the initiation of ATP-induced steroidogenesis in BAFC and its ability to sustain stimulatory levels.
[Materials & Methods] BAFC were aseptically isolated from fresh bovine adrenal cortex and were cultured in Ham's F -12 medium supplemented with serum and antibiotics. Three-day primary cultured cells were used for the experiments. Extracellular ATP was quantified by Luciferin-Luciferase assay. Steroidogenesis was determined fluorometrically with cortisol as a standard. cDNA was constructed from BAFC RNA and used for the qPCR templates.
[Results] ATP added to the external culture solution decreased to 11.5% in an hour. The rank order of expression in P2YR subtypes in BAFC was P2Y11 > P2Y6 > P2Y14 > P2Y2 > P2Y1 > P2Y12. No ecto-ATPDase was expressed by the BAFC, but alkaline phosphatase and 5'-nucleotidase were expressed.
[Conclusion] It is concluded that continuous extracellular ATP stimulation is required for steroidogenesis in BAFC.

3-P-256

Tight regulation of mitochondrial Ca^{2+} signal by MICU1 in pancreatic β-cells.

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Intracellular Ca^{2+} signal plays essential roles in insulin secretion from pancreatic β-cells. Although recent reports suggest that Ca^{2+} flows into the mitochondria via mitochondrial Ca^{2+} uniporter (MCU) is an important regulator of insulin secretion, mitochondrial Ca^{2+} dynamics in b-cells remain elusive due to limitations in the methods for the direct visualization analysis. Using recently developed high-performance intraorganellar Ca^{2+} indicator proteins, CEPIA, we here analyzed high glucose-induced mitochondrial Ca^{2+} dynamics in isolated mouse islets and insulin-secreting MIN6 cells. Unexpectedly, high glucose stimulation failed to evoke a large amplitude of mitochondrial Ca^{2+} signals. shRNA-mediated knockdown of Micu1, which is one of the essential regulators of Ca^{2+} influx into the mitochondria, increased high glucose-induced mitochondrial Ca^{2+} signals. Micu1 knockdown caused significant decrease in insulin concentration after high glucose stimulation in MIN6 cells. Moreover, overexpression of MCU also increased mitochondrial Ca^{2+} signals and induced cell death. These results indicate that in b-cells, mitochondrial Ca^{2+} signals are tightly regulated by Micu1 during high glucose condition and aberrant regulation causes pancreatic β-cell dysfunction.

3-P-257

Intracellular signal transduction of melanin concentrating hormone receptors expressed in the adenohypophysis of a basal actinopterygian fish, *Polypterus senegalus*

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Melanin concentrating hormone (MCH) is a peptide conserved from fish to mammal, and well known as an orexigenic neuropeptide in mammals. Recently, we found MCH-immunopositive fibers in the median eminence of a basal actinopterygian fish, *Polypterus senegalus*, that is located in the phylogenetic branch of fishes and tetrapod. However, the functions of MCH on the endocrine cells in the adenohypophysis are unknown. In this study, we examined the expression of MCH receptors in the gland of this fish, and their intracellular signal transductions. Phylogenetic analysis showed that *P. senegalus* has two types of MCH receptor 1 (MCHR1) and two types of MCH receptor 2 (MCHR2), that are close to the MCH receptors of mammal or teleost. *In situ* hybridization showed that mammal-like MCHR2 (*m*-MCHR2) and teleost-like MCHR2 (*t*-MCHR2) are expressed in the endocrine cells of the pars distalis of the gland, that is homologous to the anterior pituitary gland of mammal. HEK293 cells, which were transfected with these receptors separately, showed that both MCHR2s increase intracellular Ca^{2+}, and that *t*-MCHR2, but not *m*-MCHR2, inhibits the activity of the adenylate cyclase in a concentration-dependent manner. These findings suggest that two types of the MCH receptor 2 regulate release and/or production of adenohypophyseal hormone in this fish.

3-P-258

Effect of the amount of crude drug on the pharmacological action of Kampo medicine “Boufutsushosan” ～Promoting browning of white adipose tissues in high fat diet induced obese mouse～

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Aim of the study: Bofutsushosan (BTS) is a formula comprising eighteen crude drugs in traditional Japanese Kampo medicine used for obesity and metabolic syndrome. Six type of BTS prescriptions are listed Japanese Pharmacopoeia (JP), and the total amount of crude drug is different for each. It has been reported that activation of interscapular brown adipose tissue (BAT) thermogenesis is involved in lipid metabolism mechanism of BTS. While, recent data suggest that there are two distinct types of brown fat: classical brown fat derived from a myf-5 cellular lineage and UCP1-positive cells that emerge in white fat from a non-myf-5 lineage. In this study, we investigated effect of BTS extracts on lipid metabolism.

Materials and methods: BTS prescriptions were prepared from 27.1 g (BTS-1) or 28.0 g (BTS-2) of crude drugs. Mice were reared with high fat diet containing Kampo extract for 28 days, measured body fat distribution and UCP-1 mRNA expression in inguinal white adipose tissue (WAT).

Results: The amounts of visceral fat on day 28, and was significantly lower in BTS extract-treated groups. And, The expression of UCP-1 mRNA was significantly elevated in BTS-2 than BTS-1.

Conclusions: BTS has beneficial effect on obesity and metabolic syndrome, and one of its action mechanisms is the promotion for browning of white adipose tissues, and was enhanced by BTS-2. These results suggest that an increase in the amount of crude drug may have a positive effect on the pharmacological action of BTS.

3-P-259

Glucose tolerance in mice lacking glutamate decarboxylase 67 in pancreatic β cells

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Not only in the central nervous system, γ-aminobutyric acid (GABA) is also produced in and released from pancreatic β-cells. However, physiological roles of pancreatic GABA are not fully understood. GABA is produced by two isoforms of glutamate decarboxylase GAD67 and GAD65, which are encoded by the *Gad1* and *Gad2* genes, respectively. While GABAergic neurons express both GAD67 and GAD65, pancreatic β-cells only express GAD67 in mice. In this study, we developed the mice lacking GABA production in pancreatic β-cells by deleting the *Gad1* gene from these cells, and examined the glucose tolerance in those mice. The GABA-immunoreactivity (ir) was detected in the wild-type mouse pancreatic islets. In contrast, the GABA-ir was completely eliminated in *Gad1*$^{flox/flox}$;Rip-Cre mice (*Gad1*-β KO mice), indicating the *Gad1* gene deletion from β-cells in the mice. The blood glucose levels under baseline and fasting condition were comparable between untreated *Gad1*-β KO mice and the control mice. However, under the fasting condition, the blood glucose levels were significantly lower in *Gad1*-β KO mice than in the control mice 30 min after the treatment with glucose (2 g/kg, p.o.). These results indicate that the loss of GABA production in pancreatic β-cells may promote insulin secretion and suppress the elevation in blood glucose levels.

3-P-260

The analysis of *Period1* gene expression *in vivo* and *in vitro* using a micro PMT system at the early stage in diabets

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Disturbance of clock gene expression rhythms is a risk factor for diseases such as obesity. To understand the mechanism of regulating clock gene expression rhythms *in vivo*, multiple real time recording systems are required. In present studies, we show micro PMT system detect *Per1* gene expression rhythm over 1000 times comparing with the tissue contact optical sensor system as we report previously. *Per1* expression rhythm in the back skin of freely moving mouse had a peak at CT12. At this time point, we found high *Per1* expression in whole hair root tissue using micro PMT system in STZ diabetes mouse. Elevated *Per1* expression by STZ administration was observed on day 2 when blood glucose content was around 400 mg/dl, which corresponds with the time of onset of severe diabetes. In contrast, scalp hair showed elevated *Per1* expression on day 1 when blood glucose was still low in non-diabetic condition. A drastic increase of *Per1* expression on day 1 is consistent with that of the olfactory bulb, cortex, and liver in our previous reports. These results show that our μ PMT system responds to minute changes in gene expression in freely moving mice *in vivo* and in mice hair follicles *in vitro*. Furthermore, *Per1* in the hair can be used for a marker of diabetic aggravation.

3-P-261

Senescent brain pericytes induced BBB dysfunction by their senescence-associated secretory phenotype

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Aging is associated with dysfunction of blood-brain barrier (BBB), which is formed by brain vascular endothelial cells, astrocytes and brain pericytes. Senescence associated secretory phenotype (SASP) contributes to pathogenesis of age-related neurodegenerative disease. However, it is unclear how SASP affect BBB functions. Here, we investigated cellular senescence in pericytes, especially its impact on BBB function using low passage (P2) and high passage (P7) rat brain pericytes. Barrier function of the BBB is assessed by transendothelial electronic resistance (TEER) and permeability of sodium fluorescein (Na-F) using rat brain endothelial cells (RBECs) cocultured with P2 or P7 pericytes.

In P7 pericytes compared with P2 pericytes, expression levels of β-Galactosidase, Cdkn2a and Cdkn1a mRNA, which are characteristic markers of senescent cells, were significantly increased. Significant increases in p-NF-κB, IL-6, MMP-9 and IL-1β proteins are observed in P7 pericytes. In particular, IL-6 release was markedly increased. Both P2 and P7 pericytes elevated TEER of RBECs, while P7 pericytes failed to increase TEER to the extent of P2 pericytes. Unlike P2 pericytes, P7 pericytes did not decrease the permeability of RBECs to Na-F. Our findings suggest that senescent brain pericytes exhibited SASP through activation NF-κB signaling pathway. Therefore, senescent brain pericytes could induce BBB dysfunction by their SASP.

3-P-262

Aging-induced changes in mRNA expression of dopaminergic neuron-related genes in C57BL/6 mouse brain

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Parkinson's disease (PD) is characterized by the loss of dopaminergic neurons in the substantia nigra. It is well known that PD is one of the most common age-related neurodegenerative disease and a multifactorial disorder including aging, genetic and environmental factors. Although the prevalence of PD is increased with age, it remains unclear how aging affects dopaminergic neurons and expression of proteins that contribute to PD. Here, we performed real-time PCR analysis of whole brains obtained from male C57BL/6 mice at the age of 8, 32, 52 and 108 weeks to clarify age-related changes in expression of genes involved in PD and age at the onset of age-related changes. In genes expressed in dopaminergic neurons, mRNA expression levels of Th (tyrosine hydroxylase), Slc6a3 (dopamine transporter 1, DAT1), Slc18a2 (vesicular monoamine transporter 2, VMAT2) and Drd2 (dopamine receptor D2) were decreased with age. Th and Drd2 mRNA were significantly decreased in 52-week-old mice. Among the 4 PD-related genes (Atp13a2, Lrrk2, Park2 and Pink1) examined in this study, Atp13a2 and Pink1 mRNA were significantly decreased in 52-week-old mice. These findings suggest that aging-induced changes in the mRNA expression of dopaminergic neuron-related genes may occur in middle-aged brain. These normal aging-induced changes in mRNA expression could contribute to the onset and progression of PD.

3-P-263

Involvement of alteration of CD36 expression on pilocarpine-induced salivation in mouse parotid gland

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Dry mouth is observed commonly in middle-aged and elderly patients. Although the deterioration of parotid gland (PG) function in those patients is considered, the relationship between aging and hyposecretion has been unclear. We hypothesized that the reduction in expression of fatty acid translocase FAT/CD36, which facilitates the transport of fatty acids, induced dry mouth through the hyposecretion in PG. In this study, the level of CD36 expression was detected by real-time RT-PCR in male 8- and 48-weeks BALB/c mice. Also, effect of CD36 inhibitor sulfosuccinimidyl oleate (SSO) on salivary secretion of male 48-weeks BALB/c mice was assessed. Moreover, the involvement of PG CD36 in the salivary secretion of male 48-weeks senescence-accelerated mouse (SAM) was investigated. The RNA expression level of *CD36* in PG was superior to other salivary glands in BALB/c mice. SSO reduced pilocarpine-induced salivation in BALB/c mice. Compared with SAM resistant 1 (SAMR1), the pilocarpine-induced salivation in age-matched SAM prone 1 (SAMP1) was significantly decreased. In addition, the CD36 protein expression in PG was investigated by western blotting, and the expression of CD36 of SAMP1 was significantly lower than that of SAMR1. These results suggest that the CD36 plays an important role in the aging-induced hyposecretion of PG.

3-P-264

The actin-nucleating protein Fhod1 in alveolar macrophages

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The formin family proteins are a family of actin-nucleating protein conserved in eukaryotes from yeast to human. Among them, the formin homology domain-containing protein (Fhod) subfamily of formins emerged prior to the evolution of the metazoa and duplicated into Fhod1 and Fhod3 in vertebrates. Fhod3 plays an essential role in the assembly of actin into myofibrils in cardiomyocytes. Fhod1, on the other hand, contributes to the assembly of stress fibers, contractile actin structures found in cultured non-muscle cells. However, the *in vivo* role of Fhod1 remains poorly understood. To address this issue, we replaced the Fhod1 gene in mice with a lacZ reporter gene. Histological lacZ staining revealed strong expression of Fhod1 in the lung from embryonic day 12.5. Among the lung tissues, Fhod1 is expressed abundantly in alveolar macrophages. Alveolar macrophages isolated from bronchoalveolar lavage fluids of Fhod1 KO mice showed abnormal appearance and impaired cell motility. Thus, Fhod1 appears to be required for normal function of alveolar macrophages. We will discuss roles of Fhod1 in the regulation of actin dynamics in alveolar macrophages and the maintenance of normal lung function.

3-P-265

Development of diabetic retinopathy model using retinal organoids

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Stem cell-derived retinal organoids (ROs) have been investigated for applications in regenerative medicine, retinal disease models, and the safety evaluation of compounds. Although the advent of 3D organoids has provided innovative avenues, some unresolved limitations are present in organoid research: passive diffusion of oxygen and nutrients limits organoid growth and functional gain. Vascularization might overcome the aforementioned problems because oxygen and nutrient enter into the organoid core. In this study, ROs and vascular organoids (VOs) were generated from healthy human iPS cells. By co-culturing them, we attempted to create vascular-like structures in ROs. Co-culture of ROs and vascular organoids showed type IV collagen and CD31 positive vascular-like structures in retinal organoids. When Co-culture of retinal organoids and vascular organoids were cultured under hyperglycaemic conditions, the basement membrane was thickening in VOs, occludin expression was significantly decreased, and the size of ROs themselves decreased. In conclusion, the co-culture of ROs with VOs made it possible to produce ROs with vascular-like structures, and vascularized ROs could respond to the severe diabetic retinopathy model.

3-P-266

Inhibition of endogenous H_2S production suppresses the viability of multiple myeloma cells: application of carbidopa and benserazide capable of inhibiting cystathionine-β-synthase to treatment of bortezomib-resistant multiple myeloma

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H_2S is produced by cystathionine-γ-lyase (CSE), cystathionine-β-synthase (CBS) or 3-mercaptopyruvate sulfurtransferase (3-MST) in mammals. To identify a role of H_2S in the survival of multiple myeloma (MM) cells, we evaluated effects of their inhibitors on human MM-derived KMS-11 and its bortezomib (BTZ)-resistant KMS-11/BTZ cells. Viability of KMS-11, but not KMS-11/BTZ, cells was reduced by BTZ in a range of 10-30 nM. Na_2S and GYY4173, H_2S donors, promoted proliferation of both KMS-11 and KMS-11/BTZ cells. Aminooxyacetic acid (AOAA), a CBS inhibitor, strongly suppressed cell viability in KMS-11 cells and KMS-11/BTZ cells, an effect partially reversed by GYY4173. On the other hand, inhibitors of CSE and 3-MST exhibited relatively weak cytotoxicity in those cells. Carbidopa and benserazide, known as aromatic L-amino acid decarboxylase inhibitors, reduced CBS activity, and markedly decreased cell viability in KMS-11 cells and KMS-11/BTZ cells. They also reduced phosphorylation levels of NF-κB p65 in KMS-11 cells. Our data suggest that the CBS/H_2S/NF-κB pathway plays a key role in the survival of both BTZ-sensitive and -resistant MM, and that carbidopa and benserazide could be seeds for the development of selective CBS inhibitors, possibly applicable to treatment of BTZ-resistant MM.

3-P-267

A novel VDR agonist upregulates detoxification-related genes in human iPS cell-derived intestinal organoids

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Vitamin D is an essential nutrient that can be metabolized or absorbed from the diet, which has effects on various biological processes via the vitamin D receptor (VDR), a member of the nuclear hormone receptor superfamily of ligand-inducible transcription factors. To date, a number of VDR ligands that can activate this receptor have been developed, examples of which include vitamin D_3 derivatives that have been reported to have effects on inflammation and the cholesterol synthesis regulation in intestinal epithelium.

In the present study, we investigated the effects of a novel synthetic VDR agonist on pharmacokinetic gene expression using human iPS cell-derived intestinal organoids. Compared with vitamin D_3 treatment, the synthetic VDR agonist was found to increase the expression and activity of the drug-metabolizing enzyme CYP3A4, an indicator of intestinal functional maturation. Moreover, the the synthetic VDR agonist specifically increased the expression of the detoxification enzyme *UGT1A* and excretion transporter *MRP2*. These results suggest that our novel synthetic VDR agonist promotes the intestinal defense system.

3-P-268

Analysis of translation factors using public database of breast cancer patients.

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Breast cancer is originated from cancer stem cells (CSCs). Since CSCs are responsible for the chemoresistance and cancer relapse, it is essential to develop novel therapeutic drugs for the eradication of CSC. We have previously reported that translation factor X is involved in the proliferation of breast CSCs. In the present study, we examined the role of translation factor X in the xenograft experiments and clinical samples. First, we examined the effect of translation factor X on tumorigenicity in nude mice. Overexpression of factor X increased tumorigenicity of breast CSCs. Next, we analyzed the expression levels of translation factor X using clinical samples from patient with breast cancer. By analyzing RNA-seq data of breast cancer patients from the National Center for Biotechnology Information, translation factor X was highly expressed in breast cancer tissue samples, compared with normal tissue and early neoplasia. Finally, we analyzed cumulative survival analysis from The Cancer Genome Atlas. Kaplan-Meier analysis showed that mRNA level of translation factor X was reversely correlated with the overall survival time in patients with breast cancer. These results suggest the translation factor X can be used to predict breast cancer outcomes.

3-P-269

A neuropeptide VIP induces cell migration by activating the PI3K pathway via VPAC2 receptors

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Phosphatidylinostiol-trisphosphate (PIP_3) is involved in the formation of lamellipodium during cell migration. PI3K is activated by multiple signaling pathways through GPCRs and RTKs, and it is responsible for phosphorylation of PIP_2 to PIP_3 which is the activation site for AKT and PDK. VPAC2, also known as VIPR2, is a secretin family GPCR that binds two homologous neuropeptides with high affinity, vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP). We previously found that VIP increased migration of cancer cells, but the underlying mechanism remains unclear. Here we investigated the involvement of the PI3K pathway in VIP-induced cell migration. In HeLa cells overexpressing VPAC2, VPAC2 was abundantly localized to lamellipodia in the presence of VIP, and PIP_3 levels on the plasma membrane were increased. Conversely, VPAC2-silencing reduced VIP-induced increase in PIP_3 levels on the plasma membrane. Silencing of VPAC2 in MCF-7 cells inhibited VIP-induced cell migration. In contrast, MCF-7 cells stably expressing VPAC2 exhibited the increased lamellipodium extension and cell migration in vitro and in vivo. These findings suggest that VIP promotes cancer cell migration by enhancing PIP_3 synthesis through activation of VPAC2 receptors.

3-P-270

BDNF/TRKB axis provokes EMT progression to induce cell aggressiveness via cross-talk with cancer-associated fibroblasts in human parotid gland cancer

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The molecular features of parotid gland cancer (PGC) is not fully understood enough to develop an effective drug therapy because of the rarity. Since many human cancers in which tropomyosin receptor kinase B (TRKB) is highly expressed have poor prognosis, we here investigated the signaling by TRKB and the ligand brain-derived neurotrophic factor (BDNF) called the BDNF/TRKB pathway, in PGC cells using clinical specimens. In our primary culture system of patient-derived PGC cells and the cancer-associated fibroblasts (CAFs), the PGC cells co-cultured with CAFs showed a significant upregulation of BDNF and epithelial-mesenchymal transition. Additionally, the similar results were observed in PGC cells treated with conditioned medium from co-culture of PGC cells with CAFs. Moreover, TRK inhibitors suppressed BDNF-induced cell migration in PGC cells. In immunohistochemical and clinicopathological analyses of tumors from PGC patients, BDNF and TRKB were highly expressed in both tumor cells and stromal cells such as CAFs, and the expression level of TRKB in the PGC cells was significantly correlated with aggressive features including vascular invasion, nodal metastasis, and poor prognosis. Taken together, these data suggest that the BDNF/TRKB pathway may regulate PGC cell aggressiveness via cross-talk with CAFs, and could be a therapeutic target for PGC harboring invasive and metastatic features.

3-P-271

Macrophage differentiation and molecular mechanism in tumor microenvironment

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Macrophage differentiation and molecular mechanism in tumor microenvironment

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Macrophages regulate the innate immune system and maintain tissue homeostasis. In the tumor microenvironment, tumor-associated macrophages (TAMs) are found to promote tumor progression in several ways such as immune escape, angiogenesis, increased proliferation, and metastasis of cancer cells. In a solid tumor microenvironment, TAMs are thought to originate mostly from circulating monocytes; however, the molecular mechanism of monocyte differentiation into TAM remains unclear.

In this study, we aimed to determine intracellular signals or target molecules involved in TAM differentiation and explore novel therapy targeting it. We used THP-1 human monocyte cell line that is a useful tool to study macrophage in vitro. First, THP-1 cells were incubated with a conditional culture medium (CM) of different breast cancer cells (MDA-MB231, MDA-MB453, and MCF7). Among these cell lines, RT-PCR analysis demonstrated that the CM of MDA-MB231 increased the expressions of TAM markers, including CD163, CD206, CCL2, and MMP9, suggesting that THP-1 cells were polarized into TAM. Then, we determined whether TAM could promote the proliferation of MDA-MB231 breast cells. MDA-MB231 cells were incubated with TAM-derived CM, and cell proliferation was examined. We found that TAM-derived CM significantly promoted the growth of MDA-MB231 breast cells. Then, we explored the compound that inhibited the differentiation of monocytes into TAM by taking TAM markers as indicators. Further studies will show that some compounds modulate TAM differentiation.

3-P-272

Azeliragon, a RAGE antagonist, suppresses cell survival and proliferation of human prostate cancer-derived LNCaP cells: Analysis of altered cell signaling

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Advanced glycation end products (AGEs), β-amyloid and high mobility group box 1 (HMGB1), a damage-associated molecular pattern (DAMP) protein, activate the receptor for AGE (RAGE), which is involved in the pathogenesis of various diseases including diabetic complications, Alzheimer's disease, and pathological pain. Clinical trials to evaluate the efficacy of azeliragon, a RAGE antagonist, in Alzheimer's disease patients are still ongoing. Given evidence that knockdown of RAGE by RNAi inhibits prostate tumor growth, we tested whether azeliragon could reduce viability of human prostate cancer-derived LNCaP cells. We confirmed that azeliragon at 1-10 μM blocked AGE-RAGE binding, and that systemic administration of azeliragon at 5-30 mg/kg significantly reduced allodynia following intraplantar injection of all-thiol HMGB1 in mice. In LNCaP cells, azeliragon at 1-10 μM suppressed cell viability and phosphorylation of p38 MAP kinase and NF-κB p65, known as downstream signals of RAGE, but did not upregulate RAGE. Inhibitors of p38 and NF-κB, but not an anti-HMGB1-neutralizing chicken antibody, attenuated the cell viability. Our data suggest that azeliragon suppresses cell viability through suppression of p38 and NF-κB activity downstream of RAGE in LNCaP cells, suggesting its usefulness to treat castration-resistant prostate cancer.

3-P-273

Anti-tumor activity of novel cyclic naphthalene diimide derivatives

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Telomerase is an enzyme that extends telomere sequences to the end of chromosome, and is well known to be overexpressed in more than 80% of cancer cells. Therefore, telomerase is expected as a target for developing novel anticancer drug. As the telomeric repeat sequences form characteristic G-quadruplex (G4) DNA structure, we have investigated the effects of novel compounds derived from G4-binding molecules on the tumor cells. In this study, we investigated the specificity of G4 DNA binding and the anticancer activity of cyclic naphthalene diimide derivatives (cNDIs).

Binding of various cNDIs to G4 DNA was examined using isothermal titration calorimetry, circular dichroism spectrometry, and the PCR-stop assay. All the cNDIs showed G4 DNA binding with high specificity against duplex DNA. Then inhibitory activity of tumor cell growth was also examined using WST-8 assay. Among them, one cNDI with highest activity of tumor cell growth was also showed selective inhibition of tumor cells compared to cells from normal tissues. The compound induced cell apoptosis observed in the annexin V staining and caspase activation detected by Western blotting analysis. The results suggested that cNDIs are considered to be promising as new anticancer agents with improved cancer specificity.

3-P-274

Exploration of blood lncRNA biomarkers predicting oxaliplatin-induced peripheral neuropathy

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Oxaliplatin, a chemotherapeutic agent widely used for colorectal cancer, causes chronic peripheral sensory neuropathy, which often persists long after the end of drug treatment. However, few means are available to predict such long-lasting neuropathy during drug treatment. In this study, we explore minimally invasive predictive biomarkers based on circulating long non-coding RNA (lncRNA). Because many lncRNA genes show cell-type specific expression distribution, blood content of lncRNA potentially reflects extracellular release from specific cell. Therefore, the extracellular lncRNAs released from iPS cell-derived primary sensory neurons were examined using RNA sequencing. After oxaliplatin treatment, many lncRNA levels that may be specifically expressed in the primary sensory neurons were altered. In patients recovered from peripheral sensory neuropathy within one year after the end of oxaliplatin treatment, amounts of some lncRNAs in the plasma extracellular vesicle were increased during oxaliplatin treatment, while those amounts were not increased in the patients with prolonged neuropathy for more than 1 year. Overall, these circulating lncRNAs are potentially an effective biomarker predicting long-lasting peripheral neuropathy and may help in judging the continuation of oxaliplatin treatment.

3-P-275

Atrophic effects by leukemia inhibitory factor (LIF) in 85As2 gastric cancer cell-inoculated cachexia model: *in vitro* analysis with C2C12 skeletal muscle cell line

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Cancer cachexia, a syndrome characterized by anorexia and body weight loss due to low fat-free mass levels including reduced musculature, markedly decreases the quality of life in cancer patients. Although stomach cancer patients have the highest incidence of cachexia, few experimental models for the study of stomach cancer cachexia have been established. We previously established a murine model of cancer cachexia by implantation of human stomach cell line 85As2 showing symptoms of cancer cachexia observed in cancer cachexia patients, and we found leukemia inhibitory factor (LIF) as one of the cachexia-inducing factor. In this model, plasma concentrations of LIF are increased in association with the changes of hypothalamic orexigenic and anorexigenic peptide mRNA levels, as well as decreased food intake and loss of body weight with musculature reduction. However, it is unclear whether increased levels of LIF secreted from 85As2 cells have atrophic effects to musculature. In the present study, we sought to determine the atrophic effects of LIF in *in vitro* experimental system using differentiated C2C12 cells derived from murine skeletal muscle cells. The results with the cells will be presented.

3-P-276

Cytotoxicity of arsenite in combination with bufadienolide compounds against human glioblastoma cell line U-87

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Exploring novel therapeutic approaches is critically needed for the treatment of glioblastoma, due to concerns over its invasiveness and drug resistance. Trivalent arsenic derivative (arsenite, As^{III}), and two bufadienolide compounds, arenobufagin (Areno) and gamabufotalin (Gama) have been reported to induce cytotoxicity in glioblastoma cells. Herein, the cytotoxicity of As^{III} combined with Areno or Gama was evaluated in the human glioblastoma cell line U-87. A dose-dependent cytotoxicity was observed in the cells treated by As^{III}, Areno and Gama, respectively. Enhancement of cytotoxicity was induced by As^{III} combined with Areno or Gama, and synergistic cytotoxic effects of clinically achieved concentrations of As^{III} combined with Areno were further observed. Apoptosis induction accompanied by a downregulation of proform of caspase-9 and caspase-3 was observed following the treatment with the combined regimen of As^{III} and Areno. The combined regimen also caused enhanced necrosis as evidenced by a clear increase in LDH release and propidium iodide-positive cell populations. In comparison to each single drug treatment, the combined regimen apparently downregulated the expression level of p-Akt, p-mTOR; and upregulated the expression level of LC3. Collectively, the combined regimen of As^{III} and Areno exhibited a unique multivalent cytocidal effects against U-87 cells by triggering apoptotic, necrotic and autophagic cell death, suggesting that developing a new combination regimen of As^{III} and Areno may offer benefits to patients with glioblastoma.

3-P-277

Cytotoxic effects of hellebrigenin and arenobufagin against human breast cancer cells

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To gain a novel insight into therapeutic approaches to fight against breast cancer, the cytocidal effects of hellebrigenin (Helle) and arenobufagin (Areno) were investigated in MCF-7 (ER positive) and MDA-MB-231 (ER negative) human breast cancer cell lines. Helle exhibited more potent cytotoxicity than Areno in both cancer cells, and MCF-7 cells were more susceptible to both drugs. The downregulation of the expression level of Bcl-2 and Bcl-xL, the upregulation of the expression level of Bad, and the activation of caspase-8, caspase-9, along with the cleavage of PARP were observed in Helle-treated MCF-7 cells. Helle-mediated necrosis-like phenotype and G_2/M cell cycle arrest were further observed. Upregulation of the expression level of p21 and downregulation of the expression level of cyclin D1, cyclin E1, cdc25C and survivin were observed in MCF-7 cells treated with Helle and occurred in parallel with G_2/M arrest. The addition of wortmannin or 3-MA, two well-known autophagy inhibitors, slightly but significantly rescued the cells, and further inhibited necrosis induction in Helle-treated MCF-7 cells. In addition, Helle-triggered G_2/M arrest was significantly corrected by wortmannin, suggesting autophagy induction contributed to Helle-induced cytotoxicity of breast cancer cells by modulating necrosis and cell cycle arrest. Collectively, our results suggested potential usefulness of both Helle and Areno in developing therapeutic strategies to treat patients with different types of breast cancer, especially ER-positive breast cancer.

3-P-278

Differentiation-inducing factor-1 exhibited anti-metastatic effects through suppressing tumor cell adhesion to vascular epithelial cells

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We reported that differentiation-inducing factor-1 (DIF-1) inhibited the proliferation in various cancer cells. In addition, DIF-1 prevented lung colony formation in a mouse model of metastatic melanoma by suppressing cell motility and proliferation. Although adhesion between circulating tumor cells and vascular epithelial cells is the essential process of tumor metastasis, DIF-1's effect on this process remains to be elucidated. In the present study, we investigated the anti-metastatic effects of DIF-1 using human A2058 melanoma cells, murine B16BL6 melanoma cells, human HCT116 colon cancer cells and human umbilical vein endothelial cells (HUVECs). In the *in-vitro* experiments, cancer cells labeled with an enhanced green fluorescent protein (eGFP) were treated with DIF-1 (30 μM) and cocultured with confluent HUVECs for 6 h. DIF-1 significantly inhibited cancer cell adhesion to HUVECs. To clarify the mechanism of DIF-1's action, adhesion-related genes were analyzed using flow cytometry, immunofluorescence and western blotting. Cell adhesion molecules on HUVECs such as intercellular cell adhesion molecule-1 (ICAM- 1), vascular endothelial cell adhesion molecule-1 (VCAM-1) and E-selectin were suppressed by DIF-1 treatment. Furthermore, b1 integrin and focal adhesion makers such as FAK, c-Src and Paxillin were decreased by DIF-1 treatment. In the *in-vivo* experiments, 8-week-old C57BL/6 mice were divided into groups treated with or without DIF-1, which was intragastrically administered 3 days (300 mg/kg/day) before inoculation of B16BL6 melanoma cells via teil vein. Mice were euthanized 14 days after tumor cell inoculation, and lungs were excised for analysis. DIF-1 significantly inhibited the lung colony formation quantified by counting the colony number and evaluating the expressions of GFP and melanoma-specific markers. These results suggested the possibility that DIF-1 exerts anti-metastatic effect through suppressing circulating cancer cell adhesion to blood vessels in distant metastatic site.

3-P-279

Differentiation-inducing factor-1 affects cancer-associated fibroblasts (CAF) in triple negative breast cancer through CXCLs/CXCR2 axis

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Differentiation-inducing factor-1 (DIF-1) identified in *Dictyostelium discoideum* has been reported to exhibit anti-tumor effects on several cancer cells, including breast cancer cells. Tumor progression is associated with not only cancer cells themselves but also the tumor microenvironment (TME). In particular, breast cancer tumor is dominated by stromal tissue of TME in which cancer-associated fibroblasts (CAF) are well known as one of the major components of TME. Here, we investigated whether DIF-1 could affect CAF in triple negative breast cancer (TNBC). In the *in-vivo* systems, we used a tumor-bearing mouse model in which 4T1-GFP TNBC cells were inoculated into mammary fat pad. DIF-1 was intragastrically administered 5 days a week and breast tumors were evaluated at Day 14. The immunohistochemistry showed that DIF-1 decreased the expression of a CAF's marker α-SMA. In the *in-vitro* systems, 4T1 cells and mouse primary dermal fibroblasts (DFBs) were used. C-X-C type chemokines (CXCL1, CXCL2 and CXCL5)/C-X-C type chemokine receptor 2 (CXCR2) axis acts as a communication mediator between cancer cells and CAF. In cultured 4T1 cells, DIF-1 inhibited CXCL1, CXCL2 and CXCR2 mRNA expressions. Co-culture with 4T1 cells induced an increase of CXCL1, CXCL2 and CXCL5 mRNA expressions in DFBs. Co-culture with DIF-1 treated 4T1 cells significantly suppressed the increase of CXCLs mRNA expressions. These results suggested that DIF-1 might downregulate CAF progression by inhibiting both CXCLs secretion in DFBs and CXCR2 expression in 4T1 cells.

3-P-280

Aquaporin 3-targeted cancer therapy using monoclonal antibodies

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Aquaporin-3 (AQP3), a member of the water- and small molecule-transporting protein family, is involved in inflammatory disease and cancer progression. Its role in pathogenesis has been attributed to AQP3-mediated H_2O_2 or glycerol transport.

In this study, we showed that a recently established anti-AQP3 monoclonal antibody (mAb) suppresses tumor growth in allograft mouse tumor models. Administration of the anti-AQP3 mAb increased the M1/M2 ratio of tumor-associated macrophages (TAM) and improved the mitochondrial function of T cells in the tumor microenvironment (TME). In an *in vitro* study, the anti-AQP3 mAb attenuated carcinoma cell-mediated polarization of monocytes into M2-like TAMs and enhanced the cellular energy metabolism of M1 macrophages, thereby decreasing the immunosuppressive profile of TAMs. Administration of anti-AQP3 mAb also restored the TAM-induced decrease in T cell proliferation. Macrophage depletion counteracted the antitumor effect of anti-AQP3 mAb in mouse tumor models, indicating that the primary targets of anti-AQP3 mAb are macrophages.

These data suggest that anti-AQP3 mAb suppresses tumor growth by attenuating immunosuppressive M2-like TAMs, which in turn maintains the antitumor function of T cells in the TME. Thus, the anti-AQP3 mAb is a potential cancer therapy that functions by targeting TAMs.

3-P-281

Anti-tumor effects of alpha-ray emitting antibodies for uterine serous carcinoma

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Uterine serous carcinoma (USC) is a rare but aggressive type 2 endometrial carcinoma with high recurrence rate and dismal prognosis. HER2 is a potential therapeutic target because 35-44% of USCs have HER2 overexpression or gene amplification. However, the efficacy of the anti-HER2 antibody, trastuzumab, is still limited. Astatine-211 (At-211), an alpha-ray emitting radionuclide, can deliver high doses to tumors while maintaining low doses to normal tissues due to its high linear energy transfer and short range. In this study, we investigated the pharmacological effects of At-211-armed trastuzumab (^{211}At-Trastuzumab) in human USC cell lines. ^{211}At-Trastuzumab bound to HER2-poditive USC cells in a cell number-dependent manner. These bindings were HER2-specific because an addition of non-labeled trastuzumab blocked the cell binding. ^{211}At-Trastuzumab showed a significant cytotoxicity in HER2-poditive USC cells. ^{211}At-Trastuzumab is a potential radiopharmaceutical for USC treatment.

3-P-282

miR-7-5p inhibition induces ferroptosis by increasing ROS and Fe^{2+} in clinically relevant radioresistant cells

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We have established and analyzed "clinically relevant radioresistant (CRR) cells" that can survive exposing to 2 Gy/day X-rays for more than 30 days to overcome cancer treatment resistance. CRR cells show resistance against not only radiation but also docetaxel and hydrogen peroxide (H_2O_2) which is one of the reactive oxygen species (ROS). It has been shown that CRR cells produce less Fe^{2+}, ROS, and lipid peroxidation compared to the parental cells. Furthermore, we have been reported that the expression of miR-7-5p is upregulated in CRR cells and miR7-5p inhibition loses its radioresistance. Therefore, we inhibited miR-7-5p expression in CRR cells by siRNA and investigated the change of Fe^{2+}, ROS, and lipid peroxidation. As a result, miR-7-5p inhibition decreases the amount of Fe^{2+}, ROS, and Liperfluo, which is one of the ferroptosis markers and detects lipid peroxidation. Moreover, miR-7-5p inhibition decreases the gene expression of Ferritin, the iron-binding protein, and arachidonate 12-lipoxygenase (ALOX12), the lipid oxidation enzyme. It has been reported that the overexpression of ALOX12 enhances the ROS amount and lipid peroxidation by H_2O_2 treatment. Taken together, our results suggest the possibility to overcome the treatment resistance by inhibiting miR-7-5p.