3-O-01

Characterization of mononuclear cardiomyocytes in adult murine hearts

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Characterization of mononuclear cardiomyocytes in adult murine hearts

【Background and Objective】

Mammalian adult hearts have poor regenerative ability and the injured hearts scarcely recover. However, in adult mice, mononuclear cardiomyocytes (mnCMs), which account for about 10% of total cardiomyocytes, are relatively more proliferative than polynuclear cardiomyocytes (pnCMs). Therefore, we focused on mnCMs and examined their properties.

【Methods and Results】

We tried to analyze genes that are highly expressed in mnCM group. Cardiomyocytes from adult mice were separated into mnCM and pnCM groups by single cell handling device, followed by RNA-sequence analysis. As a result, about 480 genes, whose expression was markedly increased by more than 5 folds in the mnCM group as compared with the pnCM group, were extracted. These extracted genes included 4 marker genes characteristic of immature cardiomyocytes, such as *Myl7* and *Nppa*. Next, focusing on Myl7, fluorescent immunostaining was performed, and the expression of Myl7 was observed in small myocyte population.

【Discussion】

It could be concluded that mnCMs population includes subpopulation that expresses marker genes characteristic of immature cardiomyocytes, such as Myl7. Further studies would be required to elucidate the biological function of these immature cardiomyocytes.

3-O-02

The overexpression of CGRRF1 induces hypertrophy in cultured cardiomyocytes

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The overexpression of CGRRF1 induces hypertrophy in cultured cardiomyocytes

【Background】Cardiomyocytes (CMs) are terminally differentiated cells that lose proliferative capacity immediately after birth; however, it remains to be fully elucidated how CMs exit from cell cycle and reach maturity. In this study, among the factors involved in cell cycle arrest, we focused on Cell Growth Regulator With Ring Finger Domain 1 (CGRRF 1).

【Objective】To explore the biological significance of CGRRF1 in CMs.

【Methods and Results】Real time RT-PCR analyses demonstrated that the expression of CGRRF1 mRNA was increased in murine adult hearts, compared with neonatal hearts. Next, we examined the expression of CGRRF1 mRNA in adult and neonatal CMs and found that CGRRF1 expression was remarkably upregulated in adult CMs. Since the expression level of CGRRF1 increases in cells expressing p53, neonatal rat CMs were infected with adenoviral vector expressing p53. The overexpression of p53 enhanced expression of CGRRF1 mRNA. Finally, in order to investigate the biological function of CGRRF1, we constructed adenoviral vector expressing CGRRF1. Interestingly, CGRRF1 overexpression resulted in CM hypertrophy.

【Discussion】CGRRF1 was induced in CMs during growth. The overexpression of CGRRF1 enlarged CMs, suggesting that CGRRF1 may be related to CM maturation.

3-O-03
Factors inducing final cardiomyocyte maturation in newborn mice

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Introduction

Murine ventricular cardiomyocytes (CM) are still immature at birth. Within 1 month after the birth, they are maturated through physiological hypertrophy, enrichment of sarcoplasmic reticulum and formation of t-tubules bearing abundant L-type Ca^{2+} channels. This channel is involved in Ca^{2+}-induced Ca^{2+} release that causes maturated excitation-contraction and forceful ventricular contraction. In heart, the concentration of several cytokines, growth factors and hormones are known to change after birth. However, it is unclear which of the factors are responsible for the final CM maturation in mice.

Methods

The effects of various inhibitors of receptors for these factors on final CM maturation were assessed in P1-20 mice. Their cardiac function was evaluated with echocardiogram. Morphology of t-tubules and a twitch Ca^{2+} transient were analyzed in the isolated CM.

Result

Cardiac function was significantly lower in mice treated with nintedanib, an inhibitor of FGF, PDGF and VEGF receptors, or SC-144, gp130 antagonist than control mice. The nintedanib-treated mice also exhibited smaller peak of Ca^{2+} transients. We are intending to determine which of the factors are necessary for the CM maturation by using shRNA delivered with adeno-associated virus in living mice.

3-O-04

Molecular mechanisms of diastolic dysfunction in diabetic cardiomyopathy

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The early stage of diabetes mellitus (DM)-related cardiomyopathy (DMCM) is characterized by left ventricular (LV) diastolic dysfunction. The aim of this study was to elucidate the mechanism of the defective Ca^{2+} signaling underlying diastolic dysfunction in DMCM. In the streptozotocin (STZ)-induced DMCM model mice 4 weeks after STZ treatment, diastolic function was impaired without reduction of ejection fraction. In the isolated LV myocytes from DM mice, the Ca^{2+} transient decay rate was slower than that from control. In the ventricle of DM mice, the expression level of junctophilin2 protein was significantly lower, although expression levels of $Ca_V1.2$, RyR2 and SERCA2 were the same as those of control mice, suggesting that uncoupling of dyad junction starts at the early stage. The phosphorylation level of phospholamban (p-PLN) was significantly lower. Insulin treatment recovered the p-PLN level and the relaxation rate of the isolated ventricular myocardium from DM mice. Furthermore, PKA-independent insulin/PKG signaling turned out to be required for maintaining basal p-PLN. These results indicate that the reduction of p-PLN caused by insulin signaling defect is responsible for the LV diastolic dysfunction in the early stage of DMCM.

3-O-05

Motion vector analysis of the line-patterned cardiomyocytes for assessing the toxicity of anticancer drugs on left ventricular function

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Recent findings highlight the unexpected toxicity of anticancer drugs on left ventricular (LV) function. Nevertheless, a strategy to predict the potential toxicity of drugs on LV function is still limited. In the present study, we developed a novel culture system with patterned human induced pluripotent stem cell-derived cardiomyocytes (hiPSCMs) in a 96 well format and evaluated the acute-to-chronic effects of several anticancer drugs by our system. We found that doxorubicin exhibited chronic, not acute, robust toxicity on the movement of hiPSCMs. The toxicity of molecule-targeted anticancer drugs was generally mild, however, sunitinib impaired the contraction, relaxation and synchronicity of contraction more strongly than other molecule-targeted anticancer drugs. We also identified that synchronicity of contraction was impaired only by the anticancer drugs with warning on LV toxicity. Patterned hiPSCMs also exhibited distinct, mechanism of action-based, contractile responses against various cardioactive compounds. These results collectively suggest the usefulness of patterned hiPSCMs in assessing the effect of drugs on LV function.

3-O-06

Roles of low density lipoprotein receptor-related protein 1 ligands in glaucomatous optic neuropathy

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Apolipoprotein E-containing lipoproteins (E-LPs) have crucial roles of lipid transport in the central nervous system. α2-Macroglobulin (a2M) is a pan-protease inhibitor found in plasma and cerebrospinal fluid. Both of these proteins act as ligands of low density lipoprotein receptor-related protein 1 (LRP1). We previously reported that E-LPs protected neurons via the LRP1 from degeneration induced by glutamate excitotoxicity in vitro and in vivo. It has been reported that a2M is increased in aqueous humor of glaucoma patients. Here, we demonstrated that intravitreal injection of E-LPs protected retina from degeneration caused by intravitreal injection of *N*-methyl-D-aspartate in rats, a model of glaucoma. Intravitreal administration of E-LPs also attenuated the elevated a2M level in aqueous humor of the glaucoma model. Moreover, an addition of E-LPs into medium of primary cultured retinal glia decreased a2M level through LRP1. The protective effect of E-LPs against excitotoxicity in primary cultured retinal ganglion cells was interfered by a2M, but additional treatment of E-LPs overcame the interference. These results may indicate a potential therapeutic role of an LRP1 ligand in the retina as treatments for glaucoma.

3-O-07

HDAC3 inhibition ameliorates memory function via microglial skewing to M2 in Alzheimer's disease model mice

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Amyloid β (Aβ) skews microglia to M1 phenotype and induces inflammation and neurodegeneration. On the other hand, another type of microglia, M2, shows anti-inflammatory and neurotrophic effects. We previously clarified that HDAC3 inhibition induced predominance of M2 microglia and axonal growth, and recovered locomotor function in spinal cord injured mice. Therefore, this study aimed to clarify that HDAC3 inhibition skewed to M2 microglia and restored memory function in Alzheimer's disease model mice. An HDAC3 inhibitor, RGFP966 was intraperitoneally administered to 5XFAD mice, a transgenic model of Alzheimer's disease. RGFP966 improved novel object recognition memory in 5XFAD mice. When microglia in the brain of 5XFAD mice were eliminated by intracerebroventricular administration of clophosome, the effect of RGFP966 was diminished. In cultured microglia, RGFP966 treatment skewed to M2 microglia when treated 24 h after Aβ addition. Conditioned medium was collected from RGFP966-treated microglia, which recovered Aβ-induced collapse of axonal growth cones. These results suggest that HDAC3 inhibition increased predominance of M2 microglia, recovered axonal degeneration, and ameliorated memory deficit in 5XFAD mice.

3-O-08

Effects of inflammasome activation on proliferation and differentiation of neural progenitor cells

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Enhanced production of pro-inflammatory cytokines by inflammasome activation in microglia has been shown to induce neuronal damage in Alzheimer's disease (AD). Impairment of neurogenesis has been also shown to contribute to cognitive decline in AD. Recent reports showed that inflammasome activation is induced in mouse neural progenitor cells (NPC). Thus, we determined involvement of inflammasome activation on proliferation or differentiation of NPC. Activation of inflammasomes in NPC was examined by the expression of inflammasome components (NLRP3, ASC, or caspase-1), IL -1beta, or IL-18. Proliferation of NPC was examined by MTT assay. The differentiation potential of NPC into neural cells was evaluated by NeuN expression using western blot analysis. Stimulation with TNFalpha and LPS induced inflammasome activation and production of IL-1beta and IL-18 in mouse NPC. Stimulation with TNFalpha and LPS significantly inhibited the proliferation of NPC and NeuN expression in the differentiated NPC. The treatment with either IL-1beta or IL-18 significantly inhibited NeuN expression in the cells. Thus, the inflammasome activation may inhibit proliferation and neural differentiation in mouse NPC via the enhanced production of IL-1beta or IL-18.

3-O-09

Nchinpi extract restores brain aging-related decline in neuronal expression of somatostatin and neprilysin in the hippocampus

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Neurotoxic soluble amyloid-beta 42 (Aβ) oligomers are increasingly believed to cause synaptic failure in the hippocampus, leading to memory disability, in the early stages of Alzheimer's disease (AD). Hippocampal somatostatin (SST)-neprilysin (NEP) system is known to function as a defense system against the neurotoxic Aβ oligomers, and to be compromised due to aging and AD pathogenesis. Therefore, restoration of this compromised hippocampal SST-NEP system may enable prevention of onset of AD and progression of the illness. We originally found that nobiletin-rich *Citrus reticulata* peel also known as Nchinpi did prevent animal memory defect. Consistently, our pilot clinical study suggested its beneficial potential in patients with AD, without adverse side effects, including digestive symptom. In the present study in the hippocampus of aged mice as well as in primary cultures of hippocampus neurons, we therefore evaluated the impacts of Nchinpi extract on expression of SST and NEP genes. Treatment with Nchinpi extract did coordinately facilitate SST and NEP levels in the cultured neurons, as assayed by real-time RT-qPCR and ICC; and it raised mRNA levels for both genes tested in the hippocampus, when the Kampo medicine was orally given to aged (17-month-old) mice at a dose of 0.5 g/kg/day for 14 consecutive days. IHC uncovered the ability to raise neuronal SST- and NEP-immunoreactivities. These findings thus suggest that the Nchinpi extract highly likely prevents such an age-related decline in the hippocampal SST-NEP system's function.

3-O-10
Development of tau propagation mice model

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The spread of abnormal protein in neurodegenerative diseases has been shown to be strongly correlated with clinical phenotypes, and it has been pointed out that close relationship with disease progression. Recently, abnormal proteins caused by neurological diseases have the same properties as "prion" of prion diseases. Prion or prion-like proteins may convert normal molecules into abnormal forms, proliferate and propagate between cells. Abnormal proteins such as tau or α-synuclein that accumulate in brains with dementia have been shown to propagate like prion proteins. However, the expression patterns of tau in the mouse brain are different from those in humans, and the pathogenesis in the animal model of abnormal tau propagation remains incomplete. To overcome this problem, a novel mouse showing tau expression patterns similar to those of humans was developed using genome editing techniques. We inoculated the brain of this mouse with a sarkosyl-insoluble fraction containing abnormal tau derived from tauopathy patients and examined the accumulation of tau pathologies. We also performed a detailed analysis of the relationship between the inoculation site and sites where tau accumulates abnormally by histochemical and neuronal circuitry, and elucidated the propagation mechanism of the abnormally accumulated protein. This research is expected to lead to the development of novel drugs for dementia using the novel approach of "inhibition of abnormal protein propagation".

3-O-11
Regulation of innate immune response mediated by organelle communication

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Organelles extensively communicate with each other to regulate cellular responses. The aim of the present study was to reveal the involvement of organelle communication in regulation of host defense responses. In particular, we focused on the functions and roles of organelle communication in regulation of innate immune response. The innate immune system senses pathogens and induces the release of inflammatory mediators leading to the induction of host defense responses. However, the innate immune system often induces an aberrant inflammatory response, which causes severe tissue damage. Therefore, understanding the mechanisms underlying the regulation of innate immune system is required for identifying promising targets for developing anti-inflammatory drugs. We observed that communication among organelles such as lysosomes and mitochondria was involved in the regulation of innate immune response. We also observed that compounds targeting organelle communication-mediated innate immune response blocked inflammation-induced tissue damage. Here, we will discuss the spatial regulation of innate immune response and anti-inflammatory effects of compounds targeting it from the perspective of organelle communication.

3-O-12

Uptake of advanced glycation end-products mediated by scavenger receptors-1 class A in macrophage

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Advanced glycation end-products (AGEs), especially toxic AGEs derived from glyceraldehyde (AGE -2) and glycolaldehyde (AGE-3), are biologically reactive compounds. Accumulated evidence suggests that the AGEs are associated with both microvascular and macrovascular complications in diabetic mellitus. Macrophages are reported to remove extracellular AGEs from tissues via scavenger receptors, leading to the progression of atherosclerosis. In the present study, we found that AGE-2 and AGE-3 enhanced their own endocytic uptake by RAW264.7 mouse macrophage-like cells. An antibody against scavenger receptors-1 class A (SR-A, CD204) significantly prevented toxic AGEs uptake. In contrast, none of neutralizing antibody against LOX-1, CD163, CD206, RAGE and CD36 has inhibitory activity on uptake of toxic AGEs. In addition, we showed that algae-derived fucoidan or carrageenan and artificially-produced dextran sulfate which is known as an SR-A antagonistic ligand, but not the others sulfated polysaccharide, including heparin, chondroitin sulfate and hyaluronic acid, has inhibitory activities on uptake of toxic AGEs. These findings suggest that the SR-A is partially involved in the toxic AGEs uptake.

3-O-13

Macrophage-selective inhibitory effect of Kikyo-to, a Japanese traditional kampo medicine, on the viability of mouse macrophage RAW264.7 cells.

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Kikyo-to is a kampo medicine which is composed of Platycodi Radix (PR) and Glycyrrhizae Radix (GR), and used for Pharyngitis. As previous research in our laboratory has found the possibility that kikyo-to could influence cell viability of RAW264.7, we examined the effect of kikyo-to in detail.
Decoction of both crude PR and GR, that is kikyo-to (K-D), or each crude herbal medicine, or aqueous solution of extract granule preparation of kikyo-to (K-G) were exposed to RAW264.7 mouse macrophage-derived cells, CACO-2 human colorectal cancer cells and K562 human blood cancer cells. Viability of these cells were evaluated after exposure of various dilution of these preparations.
K-D decreased cell viability of RAW264.7 in a time and concentration-dependent manner and K-G also decreased cell viability of RAW264.7 in a concentration-dependent manner. In contrast, the viability of other cells did not change significantly. Decoction of GR alone decreased cell viability of RAW264.7, suggesting that components of GR may contribute to decrease in cell viability of RAW264.7, selectively. To confirm this hypothesis, further studies, functional analysis and clinical validations are needed.

3-O-14

Inter-α inhibitor proteins (IAIPs) maintain neutrophils in a resting state by regulating shape and reducing ROS production

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The plasma levels of inter-α inhibitor proteins (IAIPs) are decreased in patients with sepsis and the reduced levels correlate with increased mortality. In the present study, we examined the beneficial effects of IAIPs on human neutrophils in the treatment of sepsis. We demonstrated that IAIPs induced a spherical shape of neutrophils that was smaller in size with a smooth cellular surface in a concentration-dependent manner. IAIPs inhibited the spontaneous release of reactive oxygen species (ROS) in a concentration-dependent fashion. ROS inhibition was associated with reduction in p47phox phosphorylation on Ser328. IAIPs inhibited the NETosis that is alive consistent with the concentration-dependent inhibitory effects of IAIPs on ROS production. The inhibitory regulation of CD162 (PSGL-1) expression by IAIPs demonstrated that the suppressive effects of IAIPs on the interaction between neutrophils and other effector cells attenuates the inflammatory response. IAIPs also inhibited Zn^{2+}-induced PS expression and aggregation of erythrocytes. Our results suggest that IAIP-induced morphological changes that render neutrophils quiescent, reduce production of ROS, inhibit immunothrombosis during polymicrobial infections and erythrocytes aggregation. Thus, IAIP plays a key role in controlling neutrophil activation and eryptosis.

3-O-15

Dysregulated IL-10 transcription by Ca^{2+}-activated K^+ channel $K_{Ca}3.1$ activation in human T-cell lymphoma

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The Ca^{2+}-activated K^+ channel ($K_{Ca}3.1$) is one of key molecules that control Ca^{2+} entry, and the increase of Ca^{2+} influx generally promotes cell proliferation, migration, and cytokine production in immune cells. The anti-inflammatory cytokine, interleukin-10 (IL-10) plays a crucial role in escape from tumor immune surveillance. Recently, we discovered the dysregulated IL-10 expression and production in human T-cell lymphoma HuT-78 cells. We also showed the involvement of Smad2/3 signaling pathway in this. Both Smad2 and Smad3 were constantly activated (phosphorylated) in HuT -78 cells, Phospho-Smad2 (P-Smad2) protein expression and nuclear translocation of P-Smad2 were inhibited by the $K_{Ca}3.1$ activators. Pre-treatment with a calmodulin kinase II inhibitor, KN-62 suppressed the KCa3.1 activator-induced IL-10 down-regulation and the nuclear translocation of P-Smad2. These results suggest that the $K_{Ca}3.1$ activator-induced transcriptional repression of IL-10 in HuT-78 cells is due to the inhibition of the nuclear translocation of P-Smad2 and $K_{Ca}3.1$ activators have potential as a therapeutic option to suppress the tumor-promoting activities of IL-10 through CaMKII signaling.

3-O-16

Development of Alkyne-tagged Dopamines: Molecular Probe for Dopamine Imaging using Click Chemistry

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Dopamine is the key neuromodulator regulating brain functions and its dysfunctions leading to various neuropsychological disorders such as Parkinson's disease and schizophrenia. Despite much interest, no versatile and safe probe is available that mimic chemical and biological activity of dopamine. Here, we report the development of the first alkyne-tagged neurotransmitter, mono-*N*-propargylated dopamine (MNPD) as a versatile dopamine prove. MNPD was synthesized from dopamine with five chemical reactions, and is structurally different from endogenous dopamine only by the propargyl group ($–CH_2$ –C≡CH) on its amino group, which suggests minimal effect on the chemical properties. Importantly, the alkynyl group allows coupling of MNPD with azide group ($–N_3$) containing molecules such as fluorophores by Click-Chemistry. Indeed, MNPD was incorporated into dopaminergic neuronal cell lines as well as brain tissues, was fixed at the site, and was able to be visualized by fluorescent microscopy. Furthermore, MNPD showed dopamine-like bioactivity, suggesting MNPD can serve a true biological analog probe of dopamine. In addition to these recent results, other molecule proves, MNPD derivatives, will be discussed.

3-O-17

Rapid anxiolytic effect of electroconvulsive treatment via serotonin 5-HT4 receptor

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Anxiety disorders represent the most prevalent mental health problem. While pharmacological and cognitive-behavioral therapies are effective for these disorders, approximately one third of patients show treatment resistance. Electroconvulsive treatment (ECT) could be a candidate treatment option for the medication-resistant anxiety disorders. However its anxiolytic efficacy and mechanism of action are poorly understood. Here we characterized a potential anxiolytic effect of ECT and investigated its neuronal basis using mice. We found that a few times of ECT produced a robust anxiolytic-like behavioral effect. Further repetition of ECT additionally induced an antidepressant-like effect. Repeated ECT also strongly enhanced serotonin 5-HT_4 receptor-dependent synaptic modulation mediated by endogenous serotonin in the hippocampus. In mice lacking the 5-HT_4 receptor, the anxiolytic-like, but not antidepressant-like, effect of ECT was significantly attenuated. These results suggest a specific involvement of the enhanced 5-HT_4-dependent neuromodulation in the anxiolytic effect of ECT. Our finding suggests that ECT can be a plausible treatment option for anxiety disorders with onset of action faster than its antidepressant effect.

3-O-18

Melanin-concentrating hormone neurons contribute to dysregulation of rapid eye movement sleep in narcolepsy-model mouse

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Orexin and melanin-concentrating hormone (MCH) neurons innervate each other and have opposite effects for rapid eye movement sleep (REMs) regulation. Narcolepsy patients appear abnormal REMs behaviors such as rapid transitions into REMs. The causal role of orexin neurons in narcolepsy is well established, but that of MCH neurons remains unclear. We hypothesized that in the absence of orexins, the effects of MCH on REMs can be unbalanced, potentially contributing to aspects of abnormal REMs observed in narcolepsy. To test this hypothesis, we generated MCH-Cre::OX-KO mice and characterized sleep-wake behaviors and cataplexy with chemogenetic activation and pharmacological inhibition of MCH signaling.

In mice lacking orexins, activation of the MCH neurons also increased abnormal intrusions of REMs manifest as cataplexy and short latency transitions into REMs (SLREM). Conversely, a MCH receptor 1 antagonist, SNAP 94847, almost completely eliminated SLREM and cataplexy in OX-KO mice. These findings affirm that MCH neurons promote REMs under normal circumstances, and their activity in mice lacking orexins likely triggers abnormal intrusions of REMs into non-REMs and wake, resulting in the SLREM and cataplexy characteristic of narcolepsy.

3-O-19

Effect of exercise on functional recovery and dendritic spine morphology after focal cerebral ischemia

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The promotive effect of rehabilitation after brain stroke has been reported. However, the underlying mechanisms are not clear. The aim of this study is to reveal the effects and the mechanisms of exercise on functional recovery after cerebral ischemia. To induce reproducible permanent cerebral ischemia, male C.B-17/Icr-+/Jcl mice, aged 7weeks were subjected to middle cerebral artery occlusion (MCAO). All mice were divided into 4 groups: sham group, sham + exercise group, MCAO group and MCAO + exercise group. Exercise groups were housed in a cage with a running wheel and let free to run. Behavioral tests were performed using wire hang test and Rota-Rod test at day 14 post-ischemia. Grid walking test was performed during day 2-14 post-ischemia. Then, brains were removed, and the neuronal processes and dendritic spines of pyramidal cells in the layer 5 were visualized by microinjection with Lucifer yellow. Our preliminary results suggest that voluntary exercise can facilitate functional recovery after cerebral ischemia. We will propose the hypothesis that exercise-induced functional recovery after cerebral ischemia is related to the change of dendritic spine morphology.

3-O-20

in vivo Phenomic Screening System for Antiepileptic Drugs using Dravet Syndrome Zebrafish Model

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in vivo Phenomic Screening System for Antiepileptic Drugs using Dravet Syndrome Zebrafish Model
Epilepsy is a common chronic neurological disease affecting almost 1 million people in Japan and 50 million people worldwide. Despite availability of more than two dozen FDA-approved antiepileptic drugs, one-third of patients fail to receive adequate seizure control. Specifically, pediatric genetic epilepsies are often the most severe, debilitating and pharmaco-resistant forms of epilepsy.
The discovery of epilepsy associated genes suggests varied underlying pathologies and opens the door for development of precision medicine for each genetic epilepsy. Over 80% of patients diagnosed with Dravet syndrome carry a *de novo* mutation within the voltage-gated sodium channel gene *SCN1A* and these patients suffer with drug resistant and life-threatening seizures. Here we have developed zebrafish models for Dravet syndrome featuring inactivation of *SCN1A* with an emphasis on phenomics. we will also report recent drug screening efforts using our models with a focus on assay protocols and predictive pharmacological profiles. As the discovery and development phase rapidly moves from the lab-to-the-clinic for Dravet syndrome, it is hoped that this zebrafish-based drug discovery strategy offers a platform for how to approach any genetic epilepsy.

3-O-21

In vivo Ca^{2+} imaging analysis of β-cells with transgenic mouse expressing genetically encoded Ca^{2+} indicators

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Insulin secretion from pancreatic β-cells, which is regulated by intracellular Ca^{2+} signals, plays a crucial role in the control of blood glucose levels. Although Ca^{2+} signals in β-cells have been analyzed using in vitro/ex vivo preparations including cell lines, isolated β-cells and isolated islets, in vivo Ca^{2+} imaging has proved to be challenging. Thus, while activities of β-cells in living animals are under the influence of autonomic nervous system, hormones and other substances, Ca^{2+} signals of β-cells under physiological conditions have remained elusive. We here report an in vivo β-cells Ca^{2+} imaging method using a transgenic mouse line expressing a genetically encoded Ca^{2+} indicator YC-Nano50. Using the method, we succeeded in analyzing Ca^{2+} signals in β-cells in laparotomized mice under anesthesia, and observed synchronized Ca^{2+} oscillations in β-cells within individual islets. Furthermore, simultaneous Ca^{2+} imaging in multiple islets revealed synchronized Ca^{2+} oscillations among islets, which is the basis for pulsate insulin secretion that underlies the oscillation of blood insulin level. The present method is expected to help us gain deeper insight into the regulation of insulin secretion and diabetes.

3-O-22

Development of a chemical tag tool for near-infrared fluorescence *in vivo* imaging

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Near-infrared (NIR) fluorescence (wavelength: 700-900 nm) is useful for *in vivo* imaging because of its high tissue permeability and low contamination of autofluorescence. We developed DeQODEtag system as a chemical biology tool for specific labeling of target cells using NIR fluorescence in living animals. In this system, cells expressing DeQODEtag, which is our previously obtained single-chain variable fragment (scFv) binding to a dinitrophenyl quencher, are specifically highlighted by small-molecular QODE probes consisted of a fluorophore and the quencher. We performed a chemical structure screening and obtained a high-affinity QODE probe, namely 6SiR700-pCF3oNP. A cell line with stable expression of EGFP-DeQODEtag was prepared based on a metastatic osteosarcoma cell line LM8. In a co-culture system, our probe selectively stained DeQODEtag-expressing LM8 cells against the original LM8 cells. We also prepared mouse models bearing implanted DeQODEtag-expressing LM8 cells, and performed *in vivo* imaging of the mice with intravenously administered QODE probes. Our developed DeQODEtag system successfully performed clear visualization of target tumor cells with NIR fluorescence in living mice. Future work will focus on applications of our system to *in vivo* brain Imaging.

3-O-23

Depth profile of the nanoscale vibrations in sensory epithelium of the inner ear with a modified OCT imaging system.

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Human audition can distinguish frequencies that are only 0.2% apart as well as perceive millionfold differences in acoustic pressure. These properties stem from sound-induced nanoscale vibrations in sensory epithelium of the inner ear. The epithelium is composed of three layers; sensory hair-cell, supporting-cell, and extracellular-matrix layers. Although each layer seem to show different vibration properties in vivo, this characteristics has not yet been precisely determined. In this study, we have developed an imaging system that can record the tomography and motion of the epithelium. The underlying technique is based on a commercial optical coherence tomography (OCT) system. We equipped the system with a powerful, broadband light source. This arrangement allows us not only to achieve depth resolution of ～1.8 μm in the tomographic image but also to pursue amplitude and phase of nanoscale vibrations in each of the three layers in a live guinea pig. The system has a potential to reveal physical networks across the three layers and thereby it may contribute to identification of a force transport mechanism underlying biomechanical amplification in the cochlea.

3-O-24

Planar profile of nanoscale vibrations in sensory epithelium of the inner ear with an advanced OCT imaging system

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Audition is triggered by sound-induced nanoscale vibrations in sensory epithelium of the inner ear. The epithelium is composed of three layers; sensory hair-cell, supporting-cell, and extracellular-matrix layers. Conventional optical coherence tomography (OCT) systems planarly scan the sample to reconstitute the image and analyze the motions. However, because of the low scanning resolution, planar distribution of the vibrations inside the epithelium remains uncertain. To address this issue, we describe an advanced OCT imaging system. A high-resolution microscope and ultra-speed CMOS camera were incorporated into the system, which resulted in acquisition of a square image of 0.5 x 0.5 mm at once with resolution of 2 μm. A supercontinuum broadband light source in the system achieved depth resolution of 1.8 μm. A vibrometric technique that can stroboscopically capture the motion permits us to detect vibrations of up to 30 kHz in the object. Through a microscope equipped with the system, we recorded a planar distribution of nanoscale vibrations on the extracellular-matrixlayer in a live guinea pig.This system may contribute to clarification of a fundamental mechanism underlying hearing.

3-O-25

Brain histamine H_1 receptor occupancy measured by PET after oral administration of desloratadine and loratadine

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Objective: Some histamine H_1 receptor (H_1R) antagonists have sedative effects, caused by the blockade of histamine neural transmission. Desloratadine is a newly-marked antihistamine, but its sedative properties have not been examined by positron emission tomography (PET). We examined the brain H_1R binding potential ratio (BPR), H_1R occupancy (H_1RO) and the subjective sleepiness after oral administration of desloratadine and loratadine, the prodrug of desloratadine.

Methods: Eight healthy male volunteers underwent PET imaging with [^{11}C]doxepin after single oral administration of desloratadine (5 mg), loratadine (10 mg), or placebo in a double-blind crossover study. BPRs and H_1ROs in the cerebral cortices were calculated. Subjective sleepiness was quantified by the LARS and the SSS.

Results: BPR after loratadine administration was significantly lower than placebo ($p<0.05$), but BPR after desloratadine was not significant. There was no significant difference, however, between H_1RO after desloratadine and loratadine administration. The subjective sleepiness was not significantly different among the two antihistamines and placebo.

Conclusion: At therapeutic dose, desloratadine did not bind significantly to brain H_1Rs and did not cause significant sedation.

3-O-26

Suppression of Defender against cell death 1 leads to cardiomyocyte death

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Suppression of Defender against cell death 1 leads to cardiomyocyte death

Background/Purpose】Since cardiomyocytes lose proliferative capacity soon after birth, inhibiting cardiomyocyte death is one of the important therapeutic strategies for heart failure. The goal of this study is to identify novel genes which inhibit cell death in cardiomyocytes (CMs).

【Method/Results】We found a CM death suppressing gene Dad1, whose function in the heart has not yet been elucidated. The siRNA was used to suppress the expression of Dad1 in neonatal rat CMs (NRCMs) and evaluate cell viability. As a result, suppression of Dad1 induced cell death. In addition, suppression of Dad1 increased GRP78 expression, which is an ER stress marker, suggesting ER stress is involved in cell death. In contrast, apoptosis inhibitor z-VAD-fmk failed to suppress cell death caused by suppression of Dad1. Moreover, the expression of Stt3a, a catalytic subunit of the oligosaccharyltransferase (OST) complex which Dad1 stabilizes, was suppressed, and cardiomyocyte death was also induced by suppression of Stt3a expression.

【Conclusion】 Dad1 inhibits NRCM death and ER stress and stabilizes OST complex. Thus, enhancement of Dad1 expression could be a new therapeutic target for heart failure.

3-O-27

Comprehensive search for cardiomyocyte death-related genes using forward genetic screening

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Comprehensive search for cardiomyocyte death-related genes using forward genetic screening
[Background] Cardiomyocyte (CM) death is an important risk factor in heart failure. Though molecular mechanisms of apoptosis have been well studied, those of necrosis remain to be fully elucidated. To explore novel genes related to non-apoptotic cell death induced by reactive oxygen species, we attempt genome-wide and forward genetic screening using lentiviral shRNA library. For this purpose, we optimized experimental conditions using neonatal mouse CMs.
[Methods] After infection with a pool of lentiviral shRNA library followed by the treatment with or without H2O2 (100 μM), we prepared genomic DNA from neonatal mouse CMs. The sequence of shRNA in the lentiviral vector was analyzed using the next generation sequencer.
[Results] We determined 5359 shRNA sequences targeting approximately 1000 genes. Focusing on difference of reads between 2 groups, 12 candidate genes were obtained. 2 candidates were knocked down in neonatal mouse CMs, but cell death was not suppressed.
[Conclusions] Genome wide lentiviral shRNA screening in cardiomyocytes can be useful for determining novel genes related to cell death. However there are still some problems to be solved because of the low infection efficiency in CMs.

3-O-28

HACE1 localized in recycling endosome regulates the recycling and degradation of β_1-adrenergic receptor via Rab11 and Rab12.

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β_1-adrenergic receptor (β_1AR) is a potent regulator of cardiac function. Previously, we showed that recycling endosome (RE) is key organelle of the intracellular trafficking of β_1AR induced by ligand stimulus. Here, we found that HACE1, an E3 ligase, is localized in RE and ubiquitinates β_1AR internalized by the ligand isoproterenol (Iso). Silencing of HACE1 by siRNA resulted in a decline of β_1AR on the cell surface at steady state. Given that the ubiquitinated Rab11 functions as a recycling factor, ubiquitinated Rab11 mediated by HACE1 might recycle β_1AR to the cell surface. Ubiquitination of β_1AR was increased by Iso treatment, which was further enhanced with either bafilomycin A_1($BafA_1$), a lysosome inhibitor or MG132, a proteasome inhibitor. β_1AR was co-localized with Rab12 at RE and lysosome and interacted with each other. The co-localization at lysosome was strengthened in the presence of $BafA_1$, and the interaction was enhanced by Iso stimulus. Silencing of Rab12 led to stabilization of β_1AR with or without Iso. These data suggest that Rab11 and Rab12 were involved in the recycling and degradation of β_1AR through ubiquitination of Rab11and β_1AR mediated by HACE1 in RE, respectively.

3-O-29
Analysis of proarrhythmic potential of donepezil

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Aim: Proarrhythmic potential of donepezil was analyzed. **Methods:** Exp.1: We infused 0.1 and 1 mg/kg, i.v. of donepezil hydrochloride to the chronic atrioventricular block dogs (n=4). Exp.2: We infused 0.01, 0.1 and 1 mg/kg, i.v. of the drug to the halothane-anesthetized dogs (n=4). **Results:** Exp.1: Low dose did not alter the variables. High dose increased ventricular rate, and induced non-sustained VT in 2 animals at 14-16 min, but TdP was not observed. Exp.2: Low dose did not affect the variables. Middle dose prolonged HV and QRS without altering the other variables. High dose increased heart rate, mean blood pressure, cardiac output, ventricular contraction, but decreased the preload to left ventricle and total peripheral resistance. The dose delayed the repolarization for 5-10 min, prolonged $JT_{peak}c$ for 5-30 min, T_{peak}-T_{end} at 5 min, HV for 5-15 min and refractory period at 5 min, but shortened AH at 15 and 30 min. **Conclusion:** The high dose would have increased adrenergic tone, indirectly stimulating Ca^{2+} channel, which may explain its effects on $JT_{peak}c$ and AH, whereas it may also directly inhibit Na^{+} and K^{+} channels, increasing ventricular refractoriness. Thus, temporal discordance between direct and indirect actions may well explain proarrhythmic profile of donepezil.

3-O-30

Analysis of onset mechanism of drug-induced TdP in LQT3 patient

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Introduction: TdP occurred in LQT3 patient after switching perospirone (PER) to blonanserin (BLO) for her nocturnal delirium. PER blocked α_1-adrenoceptors with pA_2 of 2.0 ng/mL. While BLO may also have such potential, 1 μg/mL of it can inhibit hERG K^+ channel by 82%. We studied onset mechanism of TdP.

Methods: PER hydrochloride (n=4) and BLO (n=4) of 0.01, 0.1 and 1 mg/kg, i.v., were administered to the halothane-anesthetized dogs.

Results: The low dose of PER decreased total peripheral vascular resistance (TPR), but increased heart rate (HR) and cardiac output (CO), and facilitated AV conduction. The middle dose decreased mean blood pressure (MBP) and prolonged repolarization period besides those observed after the low dose. Although the high dose decreased MBP and TPR, it did not increase HR or CO. It tended to delay AV conduction, and significantly prolonged repolarization period. BLO dose-dependently decreased TPR, but increased HR, CO and cardiac contractility without affecting electrophysiological variables.

Conclusion: Lack of the reflex-mediated increase of sympathetic tone after the high dose of PER may suggest its Ca^{2+} channel inhibition suppressing TdP. BLO-induced increase of Ca^{2+} current might have induced TdP in the LQT3 patient.