Effects of class I antiarrhythmics on the guinea pig pulmonary vein myocardium

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Pulmonary veins contain a myocardial layer, whose electrical activity is considered to be involved in the genesis of atrial fibrillation. Our previous study revealed that persistent sodium current (late I_{Na}) contributes to the automaticity of the pulmonary vein myocardium. Class I antiarrhythmic drug are used for the treatment of atrial fibrillation, but effects on the automatic activity and the late I_{Na} of the pulmonary vein myocardium has not been examined. In this study, we investigated the effect of class I antiarrhythmics on the automatic activity of the isolated guinea pig pulmonary vein myocardium with microelectrode and voltage clamp experiments. All of the antiarrhythmics examined reduced the maximum rate of rise of tertiapin-induced automatic action potentials. The firing frequency and diastolic depolarization slope were decreased by aprindine, flecainide and propafenone, unaffected by pilsicainide, and increased by cibenzoline and disopyramide. The late I_{Na} induced by ramp depolarization was reduced by aprindine, flecainide and propafenone, and unaffected by pilsicainide, cibenzoline and disopyramide. These results indicate that class I antiarrhythmics have differential effects on automaticity of the pulmonary vein myocardium, and that blockade of the late I_{Na} results in inhibition of automaticity.

Electropharmacological analysis of ranolazine in vivo using the halothane-anesthetized dogs

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Introduction: Ranolazine has been shown to experimentally and clinically exert an anti-atrial fibrillatory effect, of which electropharmacological profile was not thoroughly assessed to clarify the efficacy.

Methods: Ranolazine dihydrochloride was administered at 0.3 and 3 mg/kg, i.v. to the halothane-anesthetized dogs (n=5).

Results: The low dose increased the heart rate (HR) and cardiac output (CO), whereas no significant change was observed in the mean blood pressure (MBP) or ventricular contraction. It enhanced the atrioventricular conduction, but suppressed the ventricular conduction without any change in the repolarization period. The high dose decreased the HR, MBP, ventricular contraction and CO. It prolonged the repolarization period and T_{peak}-T_{end} besides the same effects on the atrioventricular and ventricular conduction as the low dose, but it did not alter the J-T_{peak}c. It prolonged the atrial (AERP) and ventricular effective refractory period (VERP) by 21 and 29 ms, respectively, giving ΔAERP/ΔVERP of 0.72. **Conclusions:** Ranolazine has cardiodepressive action along with the ventricular depolarization and repolarization delay. Since ΔAERP/ΔVERP of dronedarone, amiodarone, bepridil and *dl*-sotalol was reported to be 1.9, 1.6, 1.0 and 1.1, respectively, ranolazine may have a limited efficacy against atrial fibrillation.

Analysis of in vivo electropharmacological effects of vanoxerine

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Introduction: While a dopamine re-uptake inhibitor vanoxerine suppresses I_{Kr}, I_{Na} and $I_{Ca,L}$ in vitro, its electropharmacological information in vivo is limited. **Methods:** Vanoxerine dihydrochloride was intravenously administered at 0.03 and 0.3 mg/kg to the halothane-anesthetized dogs (n=4) under the monitoring of cardiovascular variables. **Results:** The low dose increased the heart rate and cardiac output, whereas no significant change was observed in the mean blood pressure, ventricular contraction or pre/afterload. It prolonged the ventricular effective refractoriness without any change in ECG variables. The high dose decreased the heart rate, increased the afterload, but it did not alter the other cardiohemodynamic variables. It delayed the early as well as late repolarization, and equally prolonged the atrial and ventricular effective refractoriness. No significant change was detected in the intra-atrial, atrioventricular-nodal or intra-ventricular conductions. **Conclusions:** Cardio-stimulatory responses after the low dose could be explained by the dopamine re-uptake inhibitory mechanism. In vivo electropharmacological effects of vanoxerine may largely depend on the I_{Kr} and I_{Na} inhibition, whereas $I_{Ca,L}$ suppression may play a minor role.

Analysis of safety margin against lamotrigine-induced cardiovascular adverse events in the halothane-anesthetized dogs

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Introduction: While lamotrigine is a common antiepileptic drug, it has been reported that overdose of lamotrigine induced the hypotension, cardiac conduction delay, wide complex tachycardia and cardiac arrest. In this study, we tried to bridge the gap between lamotrigine treatment and the onset of these cardiovascular adverse events.

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

Results: The low or middle dose of lamotrigine did not alter any of the variables. The high dose significantly prolonged the PR interval for 45-60 min, QRS width at 10 and 60 min, HV interval at 15 and for 45-60 min, whereas no significant change was detected in the other variables.

Conclusion: Lamotrigine may have a wide safety margin against hemodynamic adverse events since the low dose of 0.1 mg/kg in this study would provide clinically-relevant plasma concentrations. Importantly, the atrioventricular nodal and intraventricular conduction delay indicates toxic dose of lamotrigine may inhibit Ca^{2+} and Na^{+} channels, respectively, which might partly explain clinically-observed cardiovascular adverse events of lamotrigine.

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Canstatin, a C-terminal fragment of type IV collagen α2 chain, prevents ischemia/reperfusion-induced ventricular arrhythmia in rats

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Ischemia/reperfusion (I/R) injury causes ventricular arrhythmia through inducing reactive oxygen species (ROS) and Ca^{2+} overload. We examined the effects of canstatin, a C-terminal fragment of type IV collagen $\alpha 2$ chain, on I/R-induced ventricular arrhythmia. I/R was induced by ligating left anterior descending artery for 10 min. Canstatin (20 μg/kg *i.v.*) was injected 5 min before the ligation. Ventricular arrhythmia within 10 min after reperfusion was recorded using an electrocardiogram. Neonatal rat cardiomyocytes (NRCMs) or adult rat ventricular myocytes (ARVMs) was subjected to oxygen and glucose deprivation/reoxygenation (OGD/R) in the presence or absence of canstatin (250 ng/ml). ROS production was detected by 2', 7'-dichlorofluorescin diacetate staining. Phosphorylation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) was determined by Western blotting. Canstatin significantly decreased duration of I/R-induced ventricular arrythmia without suppressing the incidence. Canstatin significantly inhibited OGD/R-induced ROS production in NRCMs and tended to inhibit OGD/R-induced phosphorylation of CaMKII in ARVMs. This study for the first time demonstrated that canstatin prevents I/R-induced ventricular arrhythmia in part through inhibiting ROS production and CaMKII activation.

Periostin prolongs action potential duration thorough inhibiting voltage-dependent Na^+ channel activity in rat ventricular myocytes

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Periostin (POSTN), a matricellular protein is related to structural remodeling of pathological heart. However, it remains to be clarified whether POSTN mediates electrical disorders of heart. Thus, we investigated the effects of POSTN on electrophysiological properties in rat ventricular myocytes. Male Wistar rats were injected with recombinant rat POSTN (64 mg/kg, i.v.) for 24 h. After electrocardiogram was recorded, ventricular myocytes were isolated. Action potential (AP) and voltage-dependent Na^+ channel (Nav) current (I_{Na}) in the isolated ventricular myocytes or neonatal rat ventricular myocytes (NRVMs) were measured by a whole cell patch-clamp technique. The QRS duration was increased in the POSTN-injected rats. The duration and time to peak of AP were prolonged with a decrease in peak amplitude of AP and I_{Na} in the isolated ventricular myocytes from POSTN-injected rats. POSTN (1 μg/ml, 24 h) suppressed the peak amplitude of I_{Na} in NRVMs. The present study for the first time demonstrated that POSTN prolongs the time to peak of AP through inhibiting Nav activity in rat ventricular myocytes. It is suggested that POSTN might cause arrhythmia via prolonging AP duration in ventricular myocytes.

Pathophysiological role of reactive astrocytes in a mouse chronic cerebral hypoperfusion model

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Chronic cerebral hypoperfusion (CCH) is manifested in various CNS diseases accompanied by cognitive impairment. We have previously reported that microglial activation induced excessive inflammatory responses, white matter injury, and resultant aggravation of cognitive impairment in a mouse CCH model with bilateral common carotid artery stenosis (BCAS). Prior to the onset of cognitive impairment, we also observed the increase in the number of GFAP-immunopositive astrocytes at day 14 after BCAS operation. Although the increase remained until day 28, the pathophysiological role of astrocytes in CCH remains to be elucidated. Here, we focused on the pathophysiological significance of the increased number of astrocytes and their regulating mechanisms in CCH. To clarify the involvement of reactive astrocytes in CCH, we checked the subtype of reactive astrocytes, a pro-inflammatory A1-like phenotype or an anti-inflammatory A2-like phenotype, and observed the increase in the expression of A2-like gene in BCAS-operated mice at day 14, whereas no significant difference was seen in the expression of A1-like gene between BCAS- and sham-operated mice. These results imply that anti-inflammatory astrocytes play important roles in the early stage of development in CCH prior to the white matter injury and cognitive impairment.

Effects of transrepression-selective liver X receptor (LXR) ligands on inflammasome activation of microglia and neural progenitor cells

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[Introduction] It is recently reported that sustained activation of inflammasomes (cytosolic protein complexes) in microglia and neural progenitor cells (NPC) may induce neuroinflammation and impaired neurogenesis in neurodegenerative diseases. While liver X receptor (LXR) activation induces transcription of lipid metabolism related genes through a mechanism called transactivation, the activation suppresses expression of genes, such as interleukin-6 (IL-6) and IL-1beta, a mechanism called transrepression. We have developed transrepression-selective LXR ligands which have anti-inflammatory actions without causing hypertriglyceridemia. We determined the effects of transrepression-selective LXR ligands on inflamasome activation of microglia and NPC.

[Materials and Methods] Activation of inflammasomes in 6-3 microglia cell clone or NPC stimulated by TNF and LPS was examined by the expression of inflammasome components (NLRP3 or caspase-1), IL-1beta, or IL-18 using Western blot analysis. The differentiation potential of NPC into neural cells was evaluated by NeuN expression using Western blot analysis. A transrepression-selective LXR ligand (AA70 or M2-76) were pretreated prior to the stimulation by TNF and LPS.

[Results] Stimulation with TNF and LPS induced caspase-1 activation and production of IL-1beta and IL-18 in microglia or NPC. Pretreatment with either AA70 or M2-76 significantly inhibited the inflammasome activation. In addition, stimulation with TNF and LPS significantly suppressed NeuN expression in the differentiated NPC. Pretreatment with either AA70 or M2-76 also significantly inhibited the suppressive effect.

[Conclusion] Pretreatment of microglia or NPC with transrepression-selective LXR ligands inhibited inflammasome activation and suppression of neural differentiation by TNF and LPS.

Signaling by hydrogen sulfide (H2S) and polysulfides (H2Sn)

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Since the identification of endogenous H_2S in the mammalian brain in 1989, studies of this molecule uncovered physiological roles in processes such as neuromodulation, vascular tone regulation, cytoprotection against oxidative stress. We previously demonstrated that H_2S induces Ca^{2+} influx in astrocytes by activating transient receptor potential (TRP) channels. During this study we found that H_2S_n activates TRP channels much more potently than does H_2S and that 3-mercaptopyruvate sulfurtransferase produces H_2S_3 and H_2S_2 that activate TRP ankyrin 1 channels Recently, we demonstrated that the chemical interaction of H_2S with nitric oxide (NO) generates H_2S_2 and H_2S_3, and that it gives a mechanism of a synergistic effect between H_2S and NO. Cysteine persulfide (Cys-SSH) together with its glutathione (GSH) counterpart (GSSH) have been proposed to be involved in redox homeostasis. We will also show that 3-mercaptopyruvate sulfurtransferase (3MST) produces Cys-SSH, GSSH, and persulfurated cysteine residues of proteins under physiological conditions together with H_2S_n and H_2S.

Induction of microsomal prostaglandin E synthase-1 contributes to neuroinflammation and neurological dysfunctions in a mouse intracerebral hemorrhage model.

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We have demonstrated that microsomal prostaglandin E synthase-1 (mPGES-1), an inducible terminal enzyme for PGE_2 synthesis, is a critical factor of stroke-reperfusion injury. In this study, we investigated the role of mPGES-1 in neuroinflammation and neurological dysfunctions observed after intracerebral hemorrhage (ICH). Collagenase was injected into the left striatum of adult mPGES-1 knockout (KO) and wild-type (WT) mice. In WT mice, mRNA and protein of mPGES-1 were significantly up-regulated in striatum and cerebral cortex after ICH. In mPGES-1 KO mice, although the hemorrhage and edema size were almost the same as WT mice, survival rate was significantly higher than WT mice. The PGE_2 production, TNF-α induction and glial activation after ICH in mPGES-1 KO brain were significantly less than those in WT brain. DAPI and TUNEL staining showed ICH-induced nuclear condensation and DNA fragmentation in mPGES-1 KO striatum were less than those in WT striatum. Furthermore, mPGES-1 KO mice showed better performance in stepping error test, rotarod test and neurological dysfunction scoring compared with the WT mice. These results suggest that mPGES-1 contributes to ICH-induced neuroinflammation, neuronal apoptosis, neurological dysfunctions and mortality through PGE_2 production. Thus, mPGES-1 may be a new therapeutic target for ICH.

Involvement of exosomes in inflammatory dopaminergic neurodegeneration.

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Parkinson's disease is one of the neurodegenerative disorders, caused by progressive degeneration of dopamine (DA) neurons in substantia nigra. Microglial activation by IFNγ/LPS treatment triggers selective loss of DA neurons in midbrain slice cultures. Exosomes are regarded as a novel factor that mediates cell-to-cell interactions. In the present study, we investigated the involvement of exosomes in DA neurodegeneration triggered by microglial activation in rat midbrain slice culture. IFNγ/LPS treatment prominently elevated exosome release from midbrain slice cultures. GW4869, a neutral sphingomyelinase 2 inhibitor, decreased exosome release and prevented IFNγ/LPS-triggered DA degeneration without the inhibition of microglial activation. To directly elucidate the involvement of activated microglial-derived exosome in DA neurodegeneration, we isolated exosomes from culture media of IFNγ/LPS-treated slices and treated them to other slice cultures. Although exosomes from control slices did not affect the survival of DA neurons, exosomes from IFNγ/LPS-treated slices significantly decreased DA neurons. Microglial activation was not triggered by exosomes from IFNγ/LPS-treated slices. These findings suggest that exosomes from activated microglia directly react to neurons and mediate DA neurodegeneration.

Local sympathetic neurons promote neutrophil egress from the bone marrow at the onset of acute inflammation

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The sympathetic nervous system plays critical roles in the differentiation, maturation, and recruitment of immune cells under homeostatic conditions, and in responses to environmental stimuli, although its role in the migratory control of immune cells remains unclear. In this study, using an advanced intravital bone imaging system, we demonstrated that the sympathetic nervous system locally regulates neutrophil egress from bone marrow for mobilization to inflammatory foci. We found that sympathetic neurons were located close to blood vessels in the bone marrow cavity; moreover, upon lipopolysaccharide (LPS) administration, local sympathectomy decreased the velocity of neutrophils, and increased the proportion of neutrophils that remained in place. We also showed that vascular endothelial cells produced C-X-C motif chemokine ligand 1 (CXCL1), which is responsible for neutrophil egress out of bone marrow. Its expression was upregulated, and was suppressed by β-adrenergic receptor blockade, resulting in inhibition of neutrophil egress into the systemic circulation. Furthermore, systemic β-adrenergic signaling blockade decreased the recruitment of neutrophils in the lung under acute systemic inflammation. Taken together, the results of this study demonstrated a new regulatory system, wherein local sympathetic nervous activation promoted neutrophil egress by inhibiting *Cxcl1* expression in bone marrow endothelial cells in a β-adrenergic signaling-dependent manner, contributing to the recruitment of neutrophils at the onset of inflammation.

Functional involvement of Na^+/Ca^{2+} exchanger type 1 in brown adipose tissue thermogenesis

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Brown adipose tissue (BAT) is a primary site for non-shivering thermogenesis in mammals. In cold temperature, β-adrenergic stimulation activates mitochondrial uncoupling protein 1 (UCP1), which consequently generates heat by uncouples of the respiratory chain. Previous report suggested that intracellular Ca^{2+} signaling induced by transient receptor potential vanilloid 2 (TRPV2) activates UCP1 in BAT thermogenesis. However, molecular mechanisms of intracellular Ca^{2+} signaling in BAT are not well characterized. We have been systematically studying the physiological functions of Na^+/Ca^{2+} exchangers, which regulate intracellular Ca^{2+} signaling. Recently, we found that Na^+/Ca^{2+} exchanger type 1 (NCX1) is abundantly expressed in interscapular BAT (iBAT) from wild-type mice. Therefore, in this study, our research interest focuses on elucidating functional involvement of NCX1 in BAT thermogenesis. Intriguingly, we observed that NCX1-heterozygous ($NCX^{+/-}$) mice did not maintain their core body temperature in cold exposure. Furthermore, UCP1 transcripts in iBAT were significantly decreased in $NCX^{+/-}$ mice compared with wild-type mice. These results suggest that NCX1 may contribute to BAT thermogenesis against cold environment.

The class III histone deacetylase SIRT1-mediated post-translational modification of Ca^{2+}-activated K^+ channel $K_{Ca}3.1$ in cancer cells

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The intermediate-conductance Ca^{2+}-activated K^+ channel $K_{Ca}3.1$ is involved in the promotion of tumor growth and metastasis, and is a potential therapeutic target for cancer. Higher $K_{Ca}3.1$ gene expression correlates with the shorter overall survival in cancer patients. Histone deacetylases (HDACs) post-translationally regulate the expression and activity of a number of proteins that play a crucial role in cancer development and progeression. We have showed that the $K_{Ca}3.1$ expression and activity are potentially reduced by the treatment with class I HDACs, HDAC2 and HDAC3 in prostate and breast cancer cell lines. Hypoxic tumor microenvironment is a common characteristic of solid cancers, and is associated with cancer metastasis and poor cancer prognosis. Hypoxia up-regulates the class III HDAC, SIRT1. Here we investigated the effect of the SIRT1 inhibitor on the $K_{Ca}3.1$ expression and activity in several types of cancer cells using real-time PCR, western blotting, flow cytometry, and voltage-sensitive fluorescence dye imaging assays. Pharmacological and siRNA-mediated inhibition of SIRT1 down-regulated $K_{Ca}3.1$ transcription and reduced its activity. These results suggest that SIRT1 may be a potential therapeutic target for $K_{Ca}3.1$-overexpressing cancers.

Ca^{2+}-activated K^+ channel $K_{Ca}2.2$ inhibiter as a possible therapy for advanced prostate cancer

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Androgen deprivation (castration) therapy andwith anti-androgens have become standardremain the clinical mainstay of treatments for metastatic and aggressive prostate cancer (PCa). Yet, these therapies are limited by the inevitable onset ofmost PCa patients will progress to castration-resistant PCa (CRPC) for several reasons such as the overexpression of androgen receptors (AR). Thus, it is necessary to develop Novel therapies are desired for the advanced PCa. Ca^{2+}-activated K^+ channels (K_{Ca}) are key molecules inregulate cancer cell behaviors including proliferation progression. We revealed the predominant expression of $K_{Ca}2.2$ in human androgen-sensitive prostate cancer cell line, LNCaP cells, using a real-time PCR, western blotting, and whole-cell patch clamp recording. The treatment with UCL1684, a $K_{Ca}2.x$ channel blocker inhibited the store-operated Ca^{2+} entry in LNCaP cells, resulting in suppressive effect on proliferation of LNCaP cells. The pharmacological or siRNA-mediated inhibition of AR for 48 hr decreased the expression level levels of $K_{Ca}2.2$ transcripts in LNCaP cells, whereas the UCL1684-induced inhibition of $K_{Ca}2.2$ activity did not affect the expression levels of AR transcripts, suggesting that $K_{Ca}2.2$ is a downstream effector of AR signaling in LNCaP cells. The short-term androgen deprivation for 48 hr decreased the $K_{Ca}2.2$ protein expression, whereas the long-term one for 96 hr increased it. Together, $K_{Ca}2.2$ might be a possible therapeutic candidate in castration-resistant PCa.

Inhibition of LAT1 activates GCN2-ATF4 survival pathway in Breast cancer cells.

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LAT1 overexpressed in many cancer cells is an attractive therapeutic target because it provides cancer cells with essential amino acids required for proliferation. JPH203, a specific competitive inhibitor for LAT1, has been reported to suppress proliferation of cancer cells. Recently, we found that anti-tumor effects of JPH203 between MDA-MB -231 cells, a model of highly malignant breast cancer cells (=TNBC) and T-47D cells(=non-TNBC) were different. To investigate the difference in JPH203 sensitivity, we analyzed global gene expression change in JPH203-responsive T-47D cells in the presence or absence of JPH203. Among them, we focused on ATF4, a master transcription factor for stress response, which activates anti-apoptotic pathway. Knockdown of ATF4 in MDA-MB-231 cells, enhanced JPH203 induced cell death. Furthermore, some reports showed that ATF4 elicite CTH, the biosynthetic enzyme for cysteine, which protects TNBC from nutrient stress. Knockdown of CTH in MDA-MB-231 cells, also enhanced growth inhibition by JPH203 treatment. These results suggest that nutrient stress caused by JPH203-treatment activated anti-apoptotic signaling via ATF4 in MDA-MB-231 cells and that CTH inhibition could be a novel way of breaking resistance to anti-LAT1 therapy in MDA-MB-231 cells.

Regulation of inflammatory cytokine response by aquaporin 5 and its pathophysiological significance in AQP5 transgenic mice

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Aquaporin-5 (AQP5) is a water-selective channel protein expressed in alveolar epithelial and submucosal gland cells, and plays an important role to maintain water homeostasis in the lung. Recently, several lines of evidence indicated that AQPs regulate not only plasma membrane water permeability, but also various cellular functions, such as cell migration and growth. In our previous study, we have found that AQP5 potentiated TNF-α-induced cytokine expression, whereas it attenuated Th2 cytokine-induced response. In the present study, we first examined the underling mechanisms involved in the attenuation by AQP5 in Th2 cytokine signaling. In AQP5-expressing cells, the IL-13-induced phosphorylation of STAT6 was lower than that in control cells. Consistent with this, phosphorylation of JAK1 and TYK2 by IL-13 in AQP5 expressing cells were considerably lower than those in control cells, suggesting that AQP5 inhibits IL-13 and tyrosine kinase complex. In addition, we have established transgenic mouse in which AQP5 is highly expressed in the lung, to investigate the pathophysiological role of modification of cytokine signaling by AQP5. The phenotypes of this transgenic mice will be also shown this presentation.

Cardiovascular functions of renal tubule- and vascular smooth muscle-specific transgenic mice expressing dominant negative TRPM7 mutant

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Magnesium ion (Mg^{2+}) is an essential divalent cation and cellular Mg^{2+} concentration is tightly regulated by various Mg^{2+} channels/transporters. Therefore, dysfunction of Mg^{2+} channels/transporters may lead to a variety of cardiovascular or neuromuscular disorders. TRPM7 is a non-selective cation channel, which predominantly permeates Mg^{2+} under physiological conditions. We generated tissue-specific transgenic mouse models expressing the dominant negative TRPM7 mutant (TRPM7DN-Tg) to study the physiological and pathophysiological mechanisms of Mg^{2+} regulation. Whole-cell patch-clamp recordings revealed that TRPM6/7 currents in HEK293 cells were almost completely attenuated by co-expression of TRPM7DN mutant. Renal tubule-specific TRPM7DN-Tg exhibited dysregulation of serum Mg^{2+} level and urinary Mg^{2+} excretion. Interestingly, in these mice, phenylephrine (PE)-induced vascular contractile responses was significantly attenuated. On the other hand, vascular smooth muscle-specific TRPM7DN-Tg showed attenuation of PE-induced contractile responses without changing serum Mg^{2+} level and urinary Mg^{2+} excretion. These results suggest that TRPM6/7 channels are tissue-dependently involved in the regulation of Mg^{2+} homeostasis and vascular contraction. Our tissue-specific TRPM7DN-Tg will be useful animal models for studying magnesium disorders.

MRTF-A regulates proliferation and survival properties of pro-atherogenic macrophages

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Atherosclerosis often results in high incidence of vascular occlusion and has been recognized as the major cause of coronary artery disease. We had previously reported that promoter polymorphism of myocardin-related transcription factor A (MRTF-A) is associated with coronary atherosclerosis. However, the contribution of MRTF-A to the development of atherosclerosis remains unclear. Macrophages are known to be important mediators of atherosclerosis. In this study, we found that MRTF-A was highly expressed in lesional macrophages in human carotid atherosclerotic plaque. To investigate the role of macrophagic MRTF-A in the pathogenesis of atherosclerosis, we generated ApoE null MRTF-A transgenic mice ($ApoE^{-/-}$/$MRTF\text{-}A^{tg/+}$), in which human MRTF-A was specifically overexpressed in monocytes/macrophages. We found that $ApoE^{-/-}$/$MRTF\text{-}A^{tg/+}$ aggravated atherosclerosis and accumulated prominent lesional macrophages in the aortic sinus. We also found that MRTF-A promoted proliferation of macrophages and mitigated apoptosis both *in vitro* and *in vivo* due to downregulation of the expression of cyclin-dependent kinase inhibitors. Taken together, our data indicated that MRTF-A contributes to the development of atherosclerosis by modulating functional properties of pro-atherogenic macrophages.

PGE_2-EP4 signaling induces blood flow recovery via accumulation of Tregs.

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Prostaglandin E_2 (PGE_2) is pro-inflammatory and immunomodulatory lipid mediator formed from PGH2 by microsomal Prostaglandin E Synthase-1 (mPGES-1). PGE_2 binds EP receptors, EP1-EP4, and induces pharmacological function. We analyzed what type of EP receptor is most important for recovery from ischemia.
Method) Male 6-8 weeks old C57Bl/6N (wild type=WT), EP4WT and EP4 receptor deficient mice (EP4KO) were used. Ischemic hind limb model was made by femoral artery ligation. Blood flow recovery was estimated by leaser Doppler images. Angiogenesis was estimated by expression of CD31, TGF-beta and SDF-1 by using immunohistichemical analysis and real time PCR. Contribution of regulatory T cells (Tregs) was estimated by immunohistochemical study and real time PCR against FOXP3 expression, that was specific transcript factor for Tregs.
Results) Expression of EP4 receptor in the ischemic muscle was enhanced compared to other EP receptors. Selective EP4 antagonist significantly suppressed recovery from ischemia compared to vehicle treated mice. Furthermore, blood flow recovery was significantly suppressed in EP4KO compared to EP4WT. Sixty seven percentage of EP4KO showed necrosis in ligated foot in contrast, no necrosis lesion was seen in EP4WT. The number of accumulated $FOXP3^+$ cells in the ischemic muscle was decreased in EP4KO compared to WT. Expression of TGF-beta and SDF-1 were suppressed in EP4KO. Moreover, EP4KO transplanted with WT-Tregs ($CD4^+CD25^+$) significantly enhanced blood flow recovery compared to EP4KO transplanted with non-Tregs ($CD4^-CD25^+$).
Those results suggested that PGE_2-EP4 signaling induced recovery from ischemia via accumulating Tregs. Highly selective EP4 agonist might be useful for treating peripheral artery disease.

Obligatory roles of caveolae in excitation-transcription coupling in vascular smooth muscle cells.

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In smooth muscle cells (SMCs), caveolin (cav)-1, an essential component of caveolae, forms Ca^{2+} microdomain accumulating voltage-dependent Ca^{2+} channels (VDCC) and ryanodine receptors (RyR). The functional coupling between VDCC and RyR causes SMC contraction, i.e. excitation-contraction (E-C) coupling. On the other hand, Ca^{2+} influx through VDCC activates Ca^{2+}/calmodulin-dependent protein kinase (CaMK), and promotes gene transcription in neurons, i.e. excitation-transcription (E-T) coupling. E-T coupling is known in SMCs, but its structural basis and physiological function are unknown. Therefore, we examined the relationships between Ca^{2+} microdomain formed by caveolae and E-T coupling in SMCs. When the mesenteric artery was depolarized, the phosphorylation of CREB in the nuclei of SMCs and induction of *c-fos* was detected. These responses were not observed in the tissue of *Cav-1* KO mouse that lacks caveolae in SMCs and those in which caveolae were destroyed by methyl b cyclodextrin. The CREB phosphorylation was significantly attenuated by a CaMKK2 inhibitor STO609 and CaMK2 inhibitor KN93. Furthermore, fluorescence imaging analyses detected a direct molecular coupling between cav1 and CaMKK2. These results suggest that caveolae accumulate Ca^{2+} channels and CaMKK2 and cause not only E-C coupling but also E-T coupling in SMCs.

The mechanism of serotonin-induced increase in intracellular Ca^{2+} and constriction via Rho kinase in rat thoracic aortas

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The mechanism of serotonin (5-HT)-induced vasoconstriction and intracellular Ca^{2+} ($[Ca^{2+}]i$) mobilization is not completely elucidated. 5-HT-induced vasoconstriction partly involves Ca^{2+}-independent activation of Rho kinase. However, the mechanism of Rho kinase activation by 5-HT is still unknown. We examined the mechanism of 5-HT-induced $[Ca^{2+}]i$ mobilization of rat aortic smooth muscle cells using microscopic fluorometry. We also investigated whether 5-HT-induced constriction in rat thoracic aortas is mediated by Rho kinase activation through Src, epidermal growth factor receptor (EGFR), and extracellular signal-regulated kinase (Erk).

5-HT induced a biphasic $[Ca^{2+}]i$ response, and the initial $[Ca^{2+}]i$ increase was attenuated by inositol triphosphate (IP_3) receptor blocker, and inhibitors of Src and phosphoinositide 3-kinase (PI3K), but not L-type Ca^{2+} channel blocker (LCBB). The second $[Ca^{2+}]i$ increase was attenuated by LCBB. Contractile response to 5-HT significantly attenuated by inhibitors of Rho kinase, Erk1/2, Src, and EGFR. These data suggest that 5-HT induces Ca^{2+} release from the endoplasmic reticulum via Src and PI3K, and subsequently extracellular Ca^{2+} influx via L-type Ca^{2+} channel, and 5-HT-induced constriction is mediated by Rho kinase activation via Src, EGFR, and Erk in rat thoracic aortas.

3-O-097 Oral Sessions

The critical roles of G-quadruplexes in neuronal developmental stages through chromatin conformational changes.

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Guanine-rich DNA and RNA can form a four-stranded structure, termed G-quadruplexes (G4) in cells. The formation of G4 is implicated in many physiological events, such as gene transcription, translation, and epigenetics. However, the presence of G4 has not been revealed in the brain. Here, we demonstrate the localization of G4 in the mouse brain by immunohistochemical analysis. In cultured mouse forebrain neurons, numerous punctate G4 immunoreactivities (G4-IR) were observed in nuclei as well as in cytoplasmic areas, including axons, dendrites, and postsynapses. Interestingly, the G4-IR in nuclei show more co-localizations with the bright spots of DAPI-positive heterochromatin clusters in cultured mature compared to immature neurons. In slices from adult mouse brain, the G4-IR were distributed throughout the brain but were particularly prominent in the hippocampus, olfactory bulb, and cerebellum. In the hippocampus, G4-IR were strongly expressed in neurons and weak in astrocytes. Consistent with the results in cultured neurons, the nuclear G4-IR were co-localized with heterochromatin in calbindin-positive mature granule cells but less in doublecortin-positive neuronal progenitor cells in the dentate gyrus. Electron microscopic immunolabeling revealed G4-IR on nucleolus-associated chromosomal domains (NADs) and cytoplasm in the adult mouse hippocampal CA1 region. These observations demonstrate the critical roles of G4 in neuronal developmental stages through chromatin conformational changes and in the cytoplasmic metabolism of RNA.

3-O-098 Oral Sessions

Identification of RNA G-quadruplexes and its role of neuronal functions in mouse brain

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G-quadruplexes (G4) are noncanonical four-stranded nucleic acid structures formed by guanine-rich sequences. Recently, a number of studies have demonstrated that RNAs containing G4 structures are involved in biological and pathological processes, including transcription, mRNA maturation, translation, and their relevance to G4-binding proteins. However, the detail function of G4 RNAs in neuron still elusive. In this study, to identify G4 RNAs in brain, we performed an RNA immunoprecipitation sequencing (RIP-seq) using a G4-specific antibody, named G4 RIP-seq. We found several mRNAs of genes that are likely relevant to neuronal function or diseases. In fact, those mRNA has several G-rich sequence motifs and we successfully determined 3D G4 structures of those motifs in vitro. Immunochemical assays of certain G4-binding protein, together with *in situ* hybridization of mRNAs suggested that cellular G4 RNA might be relevant to neuronal or neuro-physical function. In this presentation, we will discuss the recent results of this study.

3-O-099 Oral Sessions

Changes in EP3 Receptor mRNA Expression in the Brain of Mice ASD Model

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Autism spectrum disorder (ASD) is one of neurodevelopment disorders, with impairment of social behaviors as a major hallmark. Cumulating body of evidence has implicated the neuroinflammatory system as a contributing factor in the pathology of ASD. The objective of this study was to investigate the expression of prostaglandin EP3 receptor mRNA in the brain of mice ASD model. The litters born to valproic acid-treated mothers were tested for their social interaction at the age of 5-6 weeks old. Upon completion of behavioral observation, the mice were sacrificed and assessed for the expression of brain EP3 receptor mRNA. Behavioral results showed shorter duration of sniffing behavior in mice born to VPA-treated mothers. Further examination in this group of mice revealed significantly lower expression of EP3 receptor mRNA in the area prefrontal cortex and hippocampus. The present study suggests that the molecular brain mechanism involved in the arachidonic acid cascade is essential to the pathophysiology of ASD.

Cognitive dysfunction in new animal model for schizophrenia with suppression of presynaptic protein Piccolo in the prefrontal cortex of mice is involved in disturbance of neuronal network in the perirhinal cortex

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Schizophrenia, a severe psychiatric disorder, exhibits three major symptoms, including cognitive dysfunction. Piccolo, a presynaptic protein, plays a role in synaptic vesicle trafficking, and is suggested to be associated with several psychiatric disorders in the postmortem and GWAS analyses. We previously proposed that mice knocking down Piccolo in the prefrontal cortex (PFC-Piccolo-KD mice) were useful as an animal model for schizophrenia based on those face and predictive validities. In this study, we investigated the neuronal circuits that contribute to cognitive dysfunction in the new animal model.

We firstly confirmed the bidirectional connection between the PFC and ventral hippocampus (vHIP) via the perirhinal cortex (PRC) by using neuronal tracer. Cognitive dysfunction in the PFC-Piccolo-KD mice in the novel object and location recognition test was recovered by activation of the PFC-PRC circuit. Furthermore, mice knocking down Piccolo in the PRC showed impairment of cognitive memory, and its impairment was also ameliorated by activation of the PRC-vHIP circuit.

These findings suggest that disturbance of the PFC-PRC-vHIP neuronal network is involved in schizophrenia-like symptoms. Accordingly, improvement of disturbed PFC-PRC-vHIP network could be a therapeutic approach for cognitive dysfunction in schizophrenia.

Role of 5-HT_{1A} receptor in myelin damage caused by maladaptation to stress in mice

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Recent studies reported that human psychiatric disorders display oligodendroglial abnormalities and alterations in oligodendrocyte structure. Our previous studies suggested that 5-HT_{1A} receptor in the hippocampus may be involved in the protection of myelin loss induced by unadaptable excessive stress. In oligodendrocytes, adenomatous polyposis coli (APC) appears at maturation and the onset of myelination. In the present study, we investigated whether 5-HT_{1A} receptor regulate remyelination in unadaptable stress-induced myelin damage. A single exposure to restraint stress for 60 min induced a decrease in head-dipping behavior in the hole-board test. This stress response disappeared in mice that had been exposed to repeated restraint stress for 60 min/day for 14 days. In contrast, repeated exposure to restraint stress for 240 min/day for 14 days did not develop stress adaptation, and still showed a decrease in head-dipping behaviors. Immunohistochemistry analysis revealed that the expression level of APC was decreased in the dentate gyrus of the hippocampus of stress-maladaptive mice. These behavioral and biochemical changes were inhibited by chronic treatment with flesinoxan, a 5-HT_{1A} receptor agonist. The present findings indicate that activation of 5-HT_{1A} receptor may promote remyelination in stress-induced myelin damage.

Closed-loop stimulation of the medial septum terminates epilepsy seizures in rats

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Temporal lobe epilepsy with distributed hippocampal seizure foci is often intractable and secondary generalization of its seizures might lead to sudden death. Early termination of the seizures through spatially extensive hippocampal intervention is not feasible directly, due to its large size and irregular shape. In contrast, the medial septum (MS) is a promising target to govern hippocampal oscillations through its divergent connections to both hippocampi. Combining this 'proxy intervention' concept and precisely-timed stimulation, we report here that closed-loop MS electrical stimulation can quickly terminate intrahippocampal seizures and suppress their secondary generalization in a rat kindling model. Precise stimulus timing governed by internal seizure rhythms in a closed-loop manner was essential for the seizure terminating effect. Cell-type-specific optogenetic stimulation revealed that alternating activation of MS GABAergic and glutamatergic neurons within the internal seizure rhythms can underlie the seizure terminating effect. This seizure rhythm congruent MS electrical stimulation can be directly translated into clinical application.

Effects of intrastriatal memantine infusion in a mouse model of hemi-parkinsonism

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Parkinson's disease is a neurodegenerative disorder cased by loss of nigrostriatal dopaminergic neurons. For over 40 years, levodopa had been established as a gold standard for PD treatment. However, long-term treatment with levodopa is often complicated by the development of adverse effects such as abnormal involuntary movements (AIMs), referred to as levodopa-induced dyskinesia (LIDs). We here report the pharmacological effects of the intrastriatal infusion of memantine, a non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist, in a 6-OHDA-lesioned mouse model of hemiparkinsonism. Spontaneous and apomorphine-induced rotational activities with an abnormal hind limb stepping were assessed as Parkinsonian symptoms. Daily intraperitoneal injection of levodopa (15 mg/kg) was performed for 21 days, with assessing AIMs score as an index of LID development. Intrastriatal memantine infusion targeted into the right dorsal striatum, using an iPRECIO™ programmable micro infusion pump with a brain infusion kit, was examined with 4 doses (3 days/dose). Infusion of memantine significantly alleviated Parkinsonian symptoms, and it also reduced AIMs score with a dose-dependent manner. These results support the idea that over-activation of the striatal NMDA receptor function might generate both Parkinsonian symptoms and LIDs.

Development of a new treatment for hyperbilirubinemia induced psychiatric disorders; preclinical study

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【Introduction】It has been reported that hyperbilirubinemia increase the risk of psychiatric disorder including schizophrenia (Miyaoka 2000) though the molecular mechanisms are not yet well understood. Several reports show the Gunn rat, which is an animal model of congenital hyperbilirubinemia, has agitative like behaviors (i,e,. Hayashida 2009). Recently, our research group have reported that risperidone improve agitative like behaviors in Gunn rat. The risperidone strongly antagonized serotonin receptor (5HT2AR). In this study, we investigated serotonin neurotransmission in the Gunn rat.【Methods】It was investigated whether 5HTAR specific antagonist (Ketanserin) injection improve behavioral abnormality in Gunn rats. The amounts of serotonin and its metabolites in the Gunn rat were measured by high performance liquid chromatograph, furthermore, serotonergic neurons in the dorsal raphe nucleus were visualized by immunohistochemistry.【Results】Ketanserin injection improved the hyperactivity and agitation like behaviors in Gunn rats. There were significantly higher serotonin and its metabolite at the frontal cortex in the Gunn rats compared to the control rats. The immunohistochemistry showed that the number of TPH positive cells was increasing in dorsal raphe nucleus of Gunn rats.【Conclusion】The serotonergic dysfunctions in the cortical regions seem to play an important role in hyeprbilirubinaemia associated abnormal behaviors. Our study suggests that intervention with abnormal serotonergic transmission may improve symptom of hyeprbilirubinaemia associated psychosis.

Improving the accuracy of diagnosis for neurodegenerative disorders with artificial intelligence

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Objective: Diagnostic criteria for rare diseases are often revised with increased numbers of cases, because the pathogenesis of such diseases is complicated and heterogeneous. Therefore, nationwide surveillance is needed to clarify the pathogenesis of rare diseases. In this study, we applied AI (Artificial Intelligence) to diagnose neurodegenerative disorders, such as multiple system atrophy (MSA) and spinocerebellar degeneration (SCD).

Methods: We constructed an AI diagnostic system based on Chainer. After machine learning, diagnostic probability (0–1.0) was estimated for each case. Medical records of cases involving patients with MSA and SCD were provided from the Ministry of Health, Labour and Welfare. 4,949 cases involving patients with MSA and 7,073 cases involving patients with SCD between 2004 and 2008 were used for this study.

Results: Diagnostic probabilities of SND (Striata-Negra Degeneration) and OPCA (Olivo-Ponto-Cerebellar Atrophy) were estimated at 0.97 and 0.88. In contrast, the probability of SDS (Shay-Drager Syndrome) was lower than that of SND and OPCA. Diagnostic probabilities of sSCD, AD_SCD, or SP (Spastic paraplegia) were estimated at 0.95, 0.86, and 0.83. On the other hand, the probabilities of AR_SCD and Other_SCD were estimated at 0.04 and 0.03.

Conclusion: An AI diagnostic system could correctly categorize cases with SND, OPCA, sSCD, AD_SCD, and SP. Although cases involving patients with familial SCD required genetic testing, those with AD_SCD were correctly estimated by the AI diagnostic system.

Involvement of intracellular Fe^{2+} against oxidative stress in CRR cells

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To achieve more effective cancer treatment, 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. CRR cells show resistance against hydrogen peroxide (H_2O_2) that is one of the reactive oxygen species. However, the resistant mechanism to H_2O_2 has not been elucidated yet. Therefore, we investigated the involvement of iron ion in the resistant mechanism to H_2O_2 in CRR cells because iron ion has been reported to react with H_2O_2 and produce hydroxyl radical (・OH). ・OH have been shown to react with plasma membrane phospholipid and lead to cell death. Internal Fe^{2+} and ・OH amount were decreased in CRR cells compared with its parental cells. In addition, expression of ferritin, which is iron-binding protein, was increased in CRR cells. No internal H_2O_2 increase and no lipid peroxidation were seen in CRR cells after 50 μM H_2O_2 treatment for 2 hours, whereas internal H_2O_2 uptake and lipid peroxidation was increased after 50 μM H_2O_2 treatment for 2 hours in parental cells. Furthermore, Pretreatment of 10 μM of $FeCl_2$ leads to more cell death after administration of 50 μM H_2O_2 in CRR cells. Administration of phospholipid also led to further cell death after 50 μM H_2O_2 treatment in CRR cells. These results suggest that intracellular Fe^{2+} content is very important against oxidative stress response in CRR cells and control of Fe^{2+} amount may be an effective option for cancer that is resistant to treatment.

Anti-leukemia activity of baicalin and baicalein, flavonoids derived from *Scutellaria baicalensis* Georgi

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Baicalin and baicalein are the flavonoids derived from the dried roots of *Scutellaria baicalensis* Georgi, known as baikal skullcap roots (Huang Qin) in the traditional Chinese medicine. Several biological activities of these two compounds have already been reported such as anti-inflammatory, anti-allergic, and choleretic activities. In this study, we investigated an anti-leukemic activity of baicalin and baicalein against a human acute myelocytic leukemia cell line, HL-60 cells. HL-60 cells were incubated with baicalin and baicalein for several days. Cell number was counted after the incubation. Both baicalin and baicalein decreased the number of cells in a dose-dependent manner. Baicalein, a deglycosylated form of baicalin showed intense suppressive effects at lower concentrations than baicalin. Since both baicalin and baicalein showed anti-leukemic activities within a short time, we assessed the killing activities of these two compounds using a CCK-8 kit. Both baicalin and baicalein showed killing activities at higher concentrations within 24 h. Observation under a microscope of the cells incubated with these two compounds for 3 h revealed deformation of the cells i.e. abnormal cell shapes such as swelling and blebbing, suggesting that the cells exposed to these compounds went into necrosic cell death. We still continue to investigate the precise mode of action.

Baicalein disturbs the morphological plasticity and motility of breast adenocarcinoma cells depending on the tumour microenvironment.

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During tumor invasion, cancer cells change their morphology and mode of migration based on communication with the surrounding environment. Numerous studies have indicated that paracrine interactions from non-neoplastic cells impact the migratory and invasive properties of cancer cells. Thus, these interactions are potential targets for anticancer therapies. In this study, we showed that the flavones member baicalein suppresses the motility of breast cancer cells that is promoted by paracrine interactions. First, we identified laminin-332 (LN-332) as a principle paracrine factor in conditioned medium from mammary epithelium-derived MCF10A cells that regulates the morphology and motility of breast adenocarcinoma MDA-MB-231 cells. Then, we carried out a morphology-based screen for small compounds, which showed that baicalein suppressed the morphological changes and migratory activity of MDA-MB-231 cells that were induced by conditioned medium from MCF10A cells and LN-332. We also found that baicalein caused narrower and incomplete lamellipodia formation in conditioned medium-treated MDA-MB-231 cells, although actin dynamics downstream of Rho family small GTPases were unaffected. These results suggest the importance of mammary epithelial cells in the cancer microenvironment promoting the migratory activity of breast adenocarcinoma cells and show a novel mechanism through which baicalein inhibits cancer cell motility.

An anti-CD133 monoclonal antibody CMab-43 exerts anti-tumor and anti-metastasis activities for colon cancers

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Background: Cancer stem cells contribute to tumorigenesis, metastasis, and chemoresistance. A pentaspan membrane glycoprotein CD133 has been used for the isolation of stem-like cells from several cancers.
Purpose: In this study, we aimed to develop sensitive and specific anti-CD133 mAbs, which exerts anti-tumor and anti-metastasis activities.
Methods: Cell-Based Immunization and Screening (CBIS) method was employed for the development of anti-CD133 mAbs. LN229/CD133 glioblastoma cells were immunized into mice, and FCM was used for the first screening. WB and IHC screenings were further performed. Human colon cancer cell lines were used for examining the anti-tumor and the anti-metastasis activities of anti-CD133 mAbs.
Results: We established a novel anti-CD133 mAb, CMab-43 (IgG_{2a}, kappa), which demonstrated a sensitive and specific reaction against colon cancer cells in FCM, WB, and IHC analyses. CMab-43 showed cancer-specific staining patterns in colon cancer tissues. Furthermore, CMab-43 significantly reduced tumor development of colon cancer cell xenografts, and inhibited experimental metastasis of colon cancer cells.
Conclusion: CMab-43 is useful for many applications and exerts anti-tumor or anti-metastasis activities. CMab-43 could be advantageous for antibody therapy against CD133-expressing colon cancers.

Antiproliferative effects of monoclonal antibodies against (pro)renin receptor in pancreatic ductal adenocarcinoma

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We previously reported that silencing of the PRR gene, which encodes the (pro)renin receptor ((P)RR) significantly reduced Wnt/β-catenin-dependent development of pancreatic ductal adenocarcinoma (PDAC). Here, we examined the effects of a panel of blocking monoclonal antibodies (mAbs) directed against the (P)RR extracellular domain on proliferation of the human PDAC cell lines PK-1 and PANC-1 in vitro and in vivo. We observed that four rat anti-(P)RR mAbs induced accumulation of cells in the G0/G1 phase of the cell cycle and significantly reduced proliferation in vitro concomitant with an attenuation of Wnt/β-catenin signaling. Systemic administration of the anti-(P)RR mAbs to nude mice bearing subcutaneous PK-1 xenografts significantly decreased tumor expression of active β-catenin and the proliferation marker Ki-67, and reduced tumor growth. In contrast, treatment with the handle region peptide of (pro)renin did not inhibit tumor growth in vitro or in vivo, indicating that the effects of the anti-(P)RR mAbs was independent of the renin–angiotensin system. These data indicate that mAbs against human (P)RR can suppress PDAC cell proliferation by hindering activation of the Wnt/β-catenin signaling pathway. Thus, mAb-mediated (P)RR blockade could be an attractive therapeutic strategy for PDAC.

Comparison of the effects of bisoprorol and atenorol on post-infarct cardiac remodeling.

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[**Objective**] Myocardial infarction and following heart failure are major causes of death in Western countries and Japan. Beta blockers are usually used in the treatment of patients with heart failure. However, all beta blockers do not show similar beneficial effects on heart failure. We examined whether bisoprorol in comparison with atenorol provides beneficial effects on post-infarct LV remodeling in rats. [**Methods and Results**] Male SD rats were subjected to left coronary artery occlusion (MI group). Bisoprorol (MI+Biso group) or atenorol (MI+Ate group) was initiated one week after operation and lasted 4 weeks. Reduction of heart rate was similar in both beta blocker-treated groups. Five weeks after MI, MI+Biso group, but not MI+Ate group, exhibited significant attenuation of LV dilatation, LV fractional shortening impairment, and LV end-diastolic pressure elevation rather than MI group. Histological analysis showed that bisoprorol attenuated myocyte hypertrophy and interstitial fibrosis in non-infarct myocardium. In addition, MI-induced increase in malondealdehyde content, an indicator of oxidative stress, was attenuated by bisoprorol in non-infarct myocardium. Furthermore, we measured autonomic nervous system activity using heart rate vulnerability. Parasympathetic nervous system activity was higher in MI+Biso group rather than MI group and MI +Ate group. [**Conclusion**] These results suggest that bisoprorol attenuates oxidative stress and mediates automonic nervous system activity, leading to attenuation of post-infarct remodeling to a greater extent than atenolol.

Development of a screening system for the compounds that induce the cell cycle activity in mammalian cardiomyocytes

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【Background and Objective】
Since mammalian cardiomyocytes exit from cell cycle immediately after birth, the regenerative activity is limited in adult mammalian hearts. The aim of this study is to construct a screening system of the compounds that induce the cell cycle activity in mammalian cardiomyocytes, using Geminin-mAG1, a Fluorescent Ubiquitination-based Cell Cycle Indicator (FUCCI) that is stabilized in S/G2/M phases.
【Methods and Results】
We generated adenovirus vector expressing the FUCCI and infected the vector in neonatal rat cardiomyocytes. The frequency of FUCCI-positive cardiomyocytes increased in a FBS concentration dependent manner, concomitant with Ki-67-positive cells. Importantly, the cells transfected with miR-294, which was reported to be involved in cardiomyocyte proliferation, exhibited increased frequency of FUCCI-positive cells. In the presence of the proteasome inhibitor MG-132 that stabilizes FUCCI, more than 90% of the cells were positively identified, which is the limitation of this screening system.
【Conclusion】
We constructed a novel screening system for the compounds that induce the cell cycle activity in cardiomyocytes, though false positive samples cannot be completely excluded.

Contribution of the loss of insulin signaling to diastolic dysfunction in the early onset of diabetic cardiomyopathy

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Left ventricular diastolic dysfunction is one of the earliest cardiac changes in the patients with diabetic cardiomyopathy (DMCM). Ca^{2+} signaling dysfunction has been shown to occur in human and animal models of DMCM. However, its molecular mechanism remains controversial. We aimed to elucidate the underlying mechanism of Ca^{2+} signaling defects in DMCM. In the type 1 diabetes mellitus (T1DM) model mice 4 weeks after injection of streptozotocin (STZ-4W), diastolic function was impaired without reduction of ejection fraction and ventricular fibrillation was not observed, which mimics the early stage of DMCM. In the ventricles of STZ-4W mice, the basal phosphorylation level of phospholamban-Ser^{16} (p-PLN) was significantly lower than that of control. Furthermore, the maintenance of basal p-PLN was found to require insulin signaling and the downstream NO/cGMP/PKG pathway in primary cultured neonatal mouse ventricular myocytes. Chronic insulin administration via sustained release implant restored the p-PLN level and diastolic function. These effects were not correlated with blood glucose level. These results indicate that the loss of cardiac insulin signaling in T1DM plays a crucial role in the impairment of Ca^{2+} cycling system and diastolic function in the early onset of DMCM.

β2-adrenergic stimulation induces Arid5a through cAMP/PKA/CREB axis to promote IL-6 upregulation

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Background

Cardiac inflammation is an exacerbation factor of heart failure. Cardiac fibroblasts (CFs) are involved in the inflammation by producing proinflammatory cytokines. We have revealed CFs expressed β2 and β3-adrenergic receptors (AR) and isoproterenol (ISO), a non-selective βAR agonist, mainly upregulated IL-6. However, it remains to be fully clarified how β-adrenergic stimulation induces IL-6 expression in CFs.

Methods

CFs were isolated from adult mice. The expression of mRNA was measured by real-time RT-PCR. The protein level was analyzed by ELISA. The activity of transcriptional factors was assessed by western blotting and ELISA-like assay.

Results

In CFs, the stimulation of β2AR with salbutamol (SAL) increased IL-6 but not that of β3AR. *β2AR*-null CFs suppressed the expression of IL-6 in response to ISO. Bucladesine, a cAMP precursor, also upregulated IL-6. Concomitant with IL-6, Arid5a, an IL-6 mRNA stabilizing factor, was induced with SAL and bucladesine. Moreover, *Arid5a* gene ablation downregulated IL-6 both in basal and SAL treated CFs. SAL activated CREB and the inhibition of PKA with H89 blocked CREB phosphorylation. Finally, 666-15, a CREB inhibitor, suppressed SAL-mediated Arid5a and IL-6 upregulation.

Conclusion

β2-adrenergic stimulation induced Arid5a through cAMP/PKA/CREB pathway and promoted IL-6 upregulation.

β2 adrenergic signaling of cardiac fibroblasts induces cardiac hypertrophy through paracrine system

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【Background】
Isoprenaline (ISO), a β adrenergic receptor (βAR) agonist, activates the β2AR signal of cardiac fibroblasts (CFs) and induces cardiac hypertrophy. The purpose of this study is to elucidate this mechanism.
【Methods & Results】
Since ISO activated PKA, a downstream signal of β2AR, in CF, we generated mice with fibroblast-specific overexpression of the PKA catalytic subunit (PKA-OE mice) and found that PKA-OE mice exhibited cardiac hypertrophy. CFs were prepared from wild-type or PKA-OE mice, designated as WT-CFs or PKA-OE-CFs, respectively. The stimulation of neonatal rat cardiomyocytes (NRCMs) with the culture medium from PKA-OE-CFs resulted in cardiomyocyte hypertrophy, suggesting that CFs produce hypertrophic paracrine factors. In response to ISO, IL-6 increased through the PKA-pathway in WT-CFs, while TNF-α and IL-1β to lesser extent. We stimulated NRCMs with the culture medium of WT-CFs or IL-6KO-CFs. Importantly, the culture medium of ISO-stimulated WT-CFs induced cardiomyocyte hypertrophy, while not that of IL-6KO-CFs, suggesting that IL-6 directly or indirectly is involved in the hypertrophic response of NRCMs to ISO.
【Conclusion】
The β2AR stimulation in CFs causes cardiac hypertrophy by producing hypertrophic factors, such as IL-6, as a paracrine mechanism. IL-6 may be a therapeutic target of cardiac hypertrophy.

Influence of Anticancer Agent on Erectile Function; Vincristine Caused Erectile dysfunction Through Endothelial Dysfunction and Injury to the Cavernous Nerve in Rats

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Objectives:

Globally, various chemotherapeutic agents are administered to patients with cancer. Analysis of the US Food and Drug Administration (FDA) Adverse Event Reporting System (AERS) database revealed that vincristine (VCR) increased the risk of erectile dysfunction (ED). Accordingly, we investigated the mechanism underlying ED in rats administered VCR.

Methods:

Twelve-week-old male Wistar-ST rats were stratified into control and VCR groups. VCR (0.1 mg/kg) was administered intraperitoneally in a single dose to rats in the VCR group. Erectile and endothelial functions were measured using ICP and isometric tension, respectively, after 4 weeks of VCR dosage administration. Expression of biomarkers for oxidative stress (NADPH oxidase and catalase), inflammation (IL-6) and endothelial repair factors (VEGF, MCP-1, and PAI-1) was also examined.

Results:

VCR-treated rats presented significantly decreased ICP/MAP ratios (VCR: 0.48 ± 0.04, control: 0.65 ± 0.06; $p < 0.01$). Relaxation responses induced by acetylcholine and electrical stimulation were decreased in the VCR group compared to those of the control group ($p < 0.05$). The expression of NADPH oxidase-1 and IL-6 mRNA in corpora cavernosa was upregulated in the rats belonging to the VCR group compared to that of the control group rats ($p < 0.01$). Catalase, VEGF, MCP-1, and PAI-1 mRNA expression in the VCR group was downregulated compared to that in the control group ($p < 0.01$).

Conclusion:

Administering the anti-cancer agent VCR resulted in ED in rats, as the oxidative stress marker NADPH oxidase-1and inflammatory cytokine IL-6 were upregulated. VCR caused damage to rat endothelial functioning and the cavernous nerve. Therefore, cancer survivors who are administered vincristine should be carefully screened for ED.

Patient-Derived Cancer Xenograft Xebrafish Model (PDXZ) and Personalized Medicine

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Personalized medicine is to enable optimal treatment at each individual patient level, and is rapidly developing as advanced cancer individualized medicine. Current personalized medicine is based on omics information, which attempts to predict pharmacotherapy responsiveness from each patient's omics. However, since personalized medicine based on patient omics information and known medical information depends on statistical prediction, big omics data for a large number of patients is required to improve the accuracy, and enormous costs and time are required. On the other hand, the National Cancer Institute (NCI) and other organizations have made rapid progress internationally with the use of the Patient-Derived Xenograft Mouse Model (PDXM) for advanced cancer precision medicine. Recently, the PDXM system using highly immunodeficient mice is widely used in the world. However, as there are several problems in PDXM, we have constructed PDX Zebrafish Model as next-generation precision medicine protocol and report its effectiveness. We have found that the PDXZ system have big advantages such as rapid quantitative analysis of drug efficacy. These clinical PDXZ system have achieved an overwhelmingly faster therapeutic drug sensitivity test than PDXM, and the predictability of postsurgical clinical drug response has been clarified.

Chronic exposure to hypoxia facilitates chemotherapy sensitivity with downregulation of MDR1.

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Chemotherapy is widely applied to various cancers. However, the tumor acquires resistance to such cytotoxic compounds. Especially, the facilitation of drug efflux often causes therapeutic failure, which is known as multidrug resistance. Multidrug resistance protein1 (MDR1, also known as p-glycoprotein) is a typical transporter relevant to the adaptive drug resistance. Rapid progression of tumors exceeds oxygen demand, resulting in a hypoxic microenvironment. It has been believed to be a cause of multidrug resistance, however, there is almost no chronic study. Thus the current study aims to reveal the chronic effects of hypoxia on sensitivity to chemotherapy using Caco2 cells and doxorubicin. Cytotoxicity of doxorubicin was smaller when cells were exposed to hypoxia in twelve hours indeed, but it was enhanced when exposed longer duration than three days with the accumulation of doxorubicin in the cells. In accordance, the expression of MDR1 was once increased in twelve hours but suppressed after one day in response to the oxygen availability. In conclusion, prolonged hypoxia should facilitate sensitivity to chemotherapy by reducing the expression of MDR1 in spite of its acute upregulation.

Establishment of orthotopic transplantation model of mouse gallbladder cancer organoid for development and efficacy evaluation of anti-tumor agents.

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Gallbladder cancer (GBC) is relatively rare worldwide. For such rare cancer, the development of animal models is more important because it is difficult to develop optimal treatments in large-scale clinical trials. Additionally, considering that immunotherapy has emerged as a promising new modality of treatment for biliary tract cancers, to develop animal models that can analyze host immune response against cancer is becoming more important. Based on these backgrounds, we aimed to establish an orthotopic GBC mouse model. To reflect genetic alterations observed in human GBC, we first developed mouse gallbladder organoids with genetic alterations in the Kras and Trp53 genes. The knockout of Trp53 gene was introduced by CRISPR-Cas9 system. These GBC organoids could create a subcutaneous tumor in wild type mice with normal anti-tumor immunity. Additionally, an orthotopic transplant model was successfully established in wild type mice. The populations of the subsets of tumor-infiltrating immune cells were able to be analyzed in both the subcutaneous tumor model and the orthotopic transplant model. In both models, the percentage of $CD8^+$ T cells was slightly decreased and that of $CD11b^+$ $Ly6G^+$ cells significantly increased with tumor growth. Finally, the treatment of orthotopic transplant model by gemcitabine significantly decreased tumor volumes. In conclusion, we developed a novel GBC mouse model by which we can analyze tumor immune response and the efficacy of anti-tumor agents.

3-O-123 Oral Sessions

Endothelial LOX-1 plays a critical role in inflammatory thrombosis

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Aim: LOX-1 is implicated in the progression of arteriosclerosis and inflammatory diseases. In this study, we explored whether LOX-1 is involved in inflammation-related thrombosis.

Methods: To analyze thrombosis, tail-bleeding time assay was performed using male C57BL/6J (WT) and LOX-1 deficient mice (LOX-1KO). Platelet counts and thrombin-antithrombin complex (TAT) were also measured. To analyze which tissue is responsible in LOX-1-supported thrombosis, tamoxifen-inducible endothelial cell-specific LOX-1KO mice (EC-LOX-1KO) were generated and employed. LOX-1 expression in tissues was analyzed by qRT-PCR. Soluble LOX-1 (sLOX-1) in mouse plasma was measured by ELISA.

Results: In normal condition, tail-bleeding time did not show significant difference between WT and LOX-1KO. Under inflammatory condition, in WT, the bleeding time was shortened in LPS injection (5 mg/kg, i.p.) group compared to the control saline group. Consequently, platelet count was decreased, and TAT level was increased in this condition. LOX-1 expression level increased in any tissues examined and plasma sLOX-1 level increased after LPS injection. On the other hand, in LOX-1KO, above LPS-dependent changes were significantly suppressed. Furthermore, EC-LOX-1KO showed similar results to LOX-1KO, suggesting that endothelial LOX-1 plays a crucial role in thrombosis under inflammatory conditions.

Conclusions: Endothelial LOX-1 promotes inflammation-related thrombosis, which suggests prothrombotic role of endothelial LOX-1 in atheromatous inflammatory lesion.

3-O-124 Oral Sessions

Effects of febuxostat on periodontitis rats

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[Objective] Periodontitis is a chronic inflammatory disease, characterized by oxidative stress and up-regulation of pro inflammatory mediators in gingival tissue. Febuxostat, a xanthine oxidase inhibitor, has been shown to exert an anti-inflammatory and antioxidant effects. The purpose of this study is to evaluate the effects of febuxostat in periodontitis rats. [Methods and Result] Male Wistar rats were divided into three groups: periodontitis group, febuxostat-treated periodontitis group, and sham-operated group. Periodontitis was induced by ligature wire insertion to gingiva around the 2nd maxillary molar and rats were then given drinking water with or without febuxostat (5 mg/kg). After 4 weeks of periodontitis, the maxilla was extracted for morphometric and histological analyses. Pro-inflammatory cytokines levels of interleukin 1β (IL1β) and tumor necrosis factor α (TNF-α) in gingiva were assessed by RT-PCR and immunohistological staining. In rats with periodontitis, ligature placement induced alveolar bone resorption and impaired glucose tolerance. In addition, IL1β and TNF-α mRNA expression in gingiva were significantly increased and these cytokine expression in interstitial cells are increased in periodontitis rats in comparison with sham-operated rats. Febuxostat significantly reduced alveolar bone resorption, blood glucose and the level of pro inflammatory cytokines. [Conclusion] We conclude that febuxostat attenuates periodontitis and related glucose intolerance.

A novel contraction mechanism by intracellular GDP in bladder smooth muscle

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Background: When we tried to elucidate the mechanism of cooling induced contraction using pig urinary bladder smooth muscle, we found the tension force was increased by intracellular GDP with dose dependent manner.
In general, triphosphate ribonucleotides such as ATP and GTP activate cellular energy sources and GTP-binding proteins, and they also lead to contraction in smooth muscle, but contraction by GDP has not been reported.
Objectives: The aim of this study is to clarify the role of intracellular GDP and the mechanism of contraction by GDP, and whether other ribonucleotides have the same action.
Methods: We used the pig urinary bladder smooth muscle. Pig tissue was obtained from the abattoir. We performed tension force measurement. Permeabilization was done by using α-toxin or β-escin.
Results: In bladder smooth muscle intact strips, no change was observed after administration of GDP. Contraction was enhanced by administration of GDP in the presence of 1 μM calcium in α-toxin permeabilized strips. GDPβS, a non-hydrolysis analog of GDP, showed no contraction in the same condition. Furthermore, the contractile effect of GDP was not observed in β-estin permeabilized strips, which enhances membrane permeability.
Conclusions: This study indicated that an intracellular GDP enhanced the contraction of bladder smooth muscle, and that calcium was essential for the contraction.

3-O-126 Oral Sessions

Effect of novel mixed anaesthesia on platelet aggregation in mice

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Anaesthesia for animal experiments is recently up-dating from an ethical point of view. Pentobarbital, the formerly used one, has been replacing by the combination of three kinds of agents; medetomidine (adrenalin α_2 agonist, 0.3 or 0.75 mg/kg), midazolam (benzodiazepine, 4 mg/kg) and butorphanol (opioid κ-agonist, 5 mg/kg). The effect of this novel mixed anaesthesia (0.3MMB or 0.75MMB) on basic animal condition is, however, yet to be clarified. In the present study, we evaluate the effect of MMB on the circulatory system, especially on platelet aggregation.

Blood samples were collected 10 min after each anaesthesia injection to ICR-male mice (6-month-old). Pentobarbital 80 mg/kg was used as a control (PENT), and combinations with medetomidine (PENT+MDT) or with butorphanol (PENT+BTP) were also tried beside 0.3MMB and 0.75MMB. Platelet aggregation was measured by the light-transmission method using platelet-rich plasma (MCM HEMA TRACER 712).

The maximal platelet aggregation induced with collagen 0.8 μg/mL were 37.2 ± 7.7, 59.2 ± 5.0 and 86.6 ± 2.9% in PENT, 0.3MMB and 0.75MMB groups (n=5, 4, 5, $p<0.01$), respectively. Similarly, ADP 5 μM-induced platelet aggregation was also significantly enhanced by MMBs. In addition, butorphanol supplement (PENT+BTP) did not affect the aggregation, while medetomidine supplement (PENT+MDT) did enhance it. There was no difference in Prothrombin Time (PT) or in Activated Partial Thromboplastin Time (APTT) between anaesthesia groups.

Platelet is reported to express α_2 receptors and to conduct weak stimulatory signals which enhance platelet activation (secondary aggregator). We need to consider that the platelet aggregation would be higher when treated with MMB anaesthesia than with conventional pentobarbital.

Establishment of anti-horse podoplanin monoclonal antibody using Cell-Based Immunization and Screening (CBIS) method

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Purpose: Podoplanin is expressed in normal tissues including renal podocytes and lymphatic endothelial cells. To investigate the expression and function of horse podoplanin (horPDPN), sensitive and specific mAbs against horPDPN are necessary. In this study, we aimed to develop useful anti-horPDPN mAbs for many applications such as flow cytometry (FCM), western blot (WB), and immunohistochemistry (IHC).

Methods: We employed a conventional immunization method using synthetic peptides or Cell-Based Immunization and Screening (CBIS) method using horPDPN-expressed mammalian cells for producing anti-horPDPN mAbs. Anti-horPDPN mAbs were screened using enzyme-linked immunosorbent assay or FCM. Established anti-horPDPN mAbs were characterized using FCM, WB, and IHC.

Results: We developed two anti-horPDPN mAbs, PMab-202 using the peptide immunization and PMab-219 using CBIS method. PMab-202 reacted with horPDPN in FCM and WB, but did not stain horPDPN in IHC. In contrast, PMab-219 detected horPDPN in not only FCM and WB, but also IHC.

Conclusion: We have successfully established mouse anti-horPDPN mAbs, PMab-202 and PMab-219. PMab-219 is applicable for FCM, WB, and IHC analyses. CBIS method could be more advantageous to establish immunohistochemistry-applicable mAbs for elucidating the pathophysiological function of horPDPN.

Voluntary wheel running improves cardiac dysfunction associated with cancer cachexia induced by human stomach cancer cell line 85As2

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Cardiovascular disorders in cancer patients with cachexia have recently become a great concern. However, the relationship between cancer cachexia and cardiac dysfunction remains unclear, due to lack of suitable models. We established a novel murine model of cancer cachexia by implantation of human stomach cancer cell line 85As2, which represent anorexia, weight loss and low fat-free mass similar to those observed in cancer patients. In this study, we evaluated cardiac functions and investigated effects of voluntary wheel running (VWR) on cachexia-induced cardiac dysfunction using this model. 85As2 human stomach cancer cells were inoculated to male BALB/c nu/nu mice, which showed a symptomatic cachexia at 2 wks after implantation. By 8 wks after implantation, severe cardiac atrophy was developed and left ventricular ejection fraction (LVEF) was markedly reduced. VWR starting from 2 to 6 wks after implantation significantly suppressed the loss of heart weight as well as general symptoms of cachexia. Moreover, LVEF significantly increased in cachexia group with VWR, compared to those without VWR. These results suggest that our 85As2 cachexia mice model could be suitable for studying cancer cachexia with cardiac dysfunction. Additionally, VWR could improve cachexia-induced cardiac dysfunction, suggesting exercise on cardiac dysfunction in cancer patients with cachexia is a possible therapeutic approach.

Analysis of onset mechanism for tyrosine kinase inhibitor imatinib induced-left ventricular diastolic dysfunction

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Introduction: Imatinib is a tyrosine kinase inhibitor used for treating various types of cancers. The other tyrosine kinase inhibitors sunitinib and dasatinib have been reported to induce diastolic dysfunction; however, such information is lacking for imatinib.

Methods: Exp. 1: Imatinib mesylate in doses of 1 and 10 mg/kg, i.v., were administered to the halothane-anesthetized dogs (n=4). Cardiovascular variables along with biomarkers reflecting myocardial injury were measured. Exp. 2: Mitochondria isolated from the rat heart (n=2) were incubated with tyrosine kinase inhibitors, of which effects on mitochondrial respiratory complexes were assessed.

Results: Exp. 1: The low dose decreased the total peripheral vascular resistance with prolonging the isovolumic relaxation time, prolonged the QTc and J-T_{peak}c, and increased AST and LDH. Moreover, the high dose suppressed the ventricular relaxation, increased the left ventricular end-diastolic volume, prolonged HV interval and increased CPK. Exp. 2: The activity of complex II were decreased by the inhibitors.

Conclusions: Imatinib may induce myocardial injury, resulting in the left ventricular diastolic dysfunction, in which mitochondrial dysfunction might play an important role.

Maresin 1, an anti-inflammatory lipid mediator, induces physiological hypertrophy in neonatal rat cardiomyocytes

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【Background】

Maresin1 (MaR1), a lipid mediator biosynthesized from docosahexaenoic acid (DHA), has both anti-inflammatory and proresolving activities. Much attention has been paid to the functional regulation by MaR1 in inflammatory cells, but not in tissue component cells. Since inflammatory reactions are involved in cardiovascular diseases, we addressed the effects of MaR1 on cardiomyocytes.

【Methods & Results】

Neonatal rat cardiomyocytes (NRCMs) were cultured with MaR1 for 48 hours. Immunofluorescent microscopic analyses using anti-sarcomeric α-actinin revealed that MaR1 increased cell surface area in a dose-dependent manner. Real time RT-PCR analyses demonstrated that the expression of the pathological hypertrophy markers, such as BNP and skeletal-actin, was not upregulated in NRCMs cultured with MaR1, indicating that MaR1-induced hypertrophy is physiological. Finally we treated NRCMs with SR3335, an ROR alpha inhibitor, because MaR1 was previously reported to utilize ROR alpha as a receptor. Importantly, SR3335 prevented the increase in cell surface area induced by MaR1.

【Conclusion】

MaR1 induces physiological hypertrophy of neonatal rat CMs through stimulating ROR alpha. MaR1 could play an important role in the tissue repair after myocardial injury.

Dad1 inhibits cardiomyocyte death by promoting cell adhesion

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[Background] Cardiomyocyte (CM) death causes the loss of CMs, resulting in heart failure. Therefore, the prevention of CM death could be a therapeutic strategy against heart failure. Defender against cell death 1 (Dad1) was reported to play anti-apoptotic roles in neural cells. Though Dad1 is highly expressed in heart, the functional roles of Dad1 remain to be elucidated. Previously, we reported that the knockdown of Dad1 induced CM death. The aim of this study is to elucidate the cytoprotective mechanism of Dad1 in CMs.

[Methods/Results] CMs were prepared from neonatal rats. The expression of Dad1 was suppressed by using siRNA. Immunofluorescent microscopic analyses demonstrated that the Dad1 knock-down CMs exhibited a round shape. Immunoblot analyses showed that the expression of N-Cadherin, a cell adhesion protein, was reduced in Dad1 knock-down CMs. Moreover, the expression of phosphorylated focal adhesion kinase (pFAK) expression, which is reported to regulate cell adhesion, was downregulated by the knockdown of Dad1, but not FAK protein. Importantly, a cell adhesion enhancer, adhesamine, prevented CM death caused by suppression of Dad1.

[Conclusion] Dad1 could protect CMs from cell death by regulating cell adhesion. Dad1 may be a novel target to prevent CM death in heart failure.

3-O-132 Oral Sessions

Impact of extracellular substrate stiffness on macrophage activation

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Increased tissue stiffness has been observed in aged vascular vessels and arteriosclerosis. Recent studies have revealed that substrate stiffness dictates macrophage activation and polarization. Thus, vascular stiffness has can potentially lead to macrophage activation that is at the center of the pathogenesis underlying chronic inflammatory diseases such as atherosclerosis. We have focused on the classical M1- and/or alternative M2-like macrophage activation on substrate whose stiffness values are relevant to normal vascular stiffness. Here we have studied the impact of the substrate stiffness on macrophage phenotypes.

In this study, we have utilized agarose gel for providing soft stiffness substrate and have found that soft substrate impairs pro-inflammatory activation of THP-1 cell under M1-promoting polarization condition. We have shown that soft substrate dictates the macrophage polarization to anti-inflammatory M2-like macrophage. Moreover, we have herein determined that peroxisome proliferator-activated receptor γ expression in macrophage on soft substrate contributes the anti-inflammatory macrophage activation.

The results shown in this study strongly suggest that extracellular substrate stiffness is a determinable factor for macrophage activation and polarization.

Resident cardiac macrophages are involved in cardioprotection through metabolic regulation of cardiomyocytes

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In Japan, the number of heart failure patients is expected to increase from now on. So, how to prevent or cure heart failure is a pressing issue. Recent studies have reported that resident macrophages in the heart maintain cardiac function. However, it isn't sufficiently understood how cardiac macrophages are involved in cardioprotection. Since heart failure involves myocardial metabolic disorders, here we hypothesized that cardiac macrophages might control myocardial metabolism by amphiregulin (AREG). Cardiomyocytes mainly oxidize fatty acids to make ATP, but under stress, they use glycolysis instead of fatty acids. This metabolic flexibility is thought to be important for maintaining myocardial homeostasis and preventing heart failure. AREG activates pyruvate dehydrogenase (PDH), an enzyme that regulates entry into the TCA cycle from the glycolysis. PDH is phosphorylated by Pdk4 and dephosphorylated by Pdp2, and dephosphorylated form is active form. The expression level of Pdk4 decreased and that of Pdp2 increased by AREG. These days suggest that AREG enhances the flow of the substrate from the glycolysis to the TCA cycle by activating PDH. Therefore, AREG regulates the metabolism via PDH in cardiomyocytes. In other words, cardiac macrophages protect cardiac function by regulating myocardial metabolism.