2-YIA-30 YIA

Zinc-aggravated M1 microglia suppress astrocytic engulfing activity via P2X7 receptors

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[AIM] M1 microglia influence astrocytic neuroprotective functions, including engulfment of cell debris. Recently, extracellular zinc has been shown to aggravate M1 phenotype in microglia through intracellular zinc accumulation and reactive oxygen species (ROS) generation. Here, we investigated whether zinc-enhanced M1 microglia affects the astrocytic engulfing activity.

[METHODS] Mouse primary astrocytes were preincubated with microglial-conditioned medium (MCM) collected from M1 microglia induced by lipopolysaccharide (LPS) after $ZnCl_2$ treatment in the presence of TPEN, a membrane permeable zinc chelator, or Trolox, a ROS scavenger, and then incubated with fluorescent latex beads. P2X7 receptors (P2X7R) mRNA level in astrocytes was measured by real-time PCR.

[RESULTS] MCM from M1 microglia increased the astrocytes bead uptake. This increased uptake activity was suppressed when MCM from LPS-induced M1 microglia pretreated with $ZnCl_2$ was applied to astrocytes, which was further abolished by TPEN and Trolox. In addition, P2X7R mRNA level was increased in astrocytes treated with MCM from M1 microglia, but not in the M1 microglia pretreated with $ZnCl_2$.

[CONCLUSION] These results suggest zinc pretreatment abolishes the ability of M1 microglia to increase the engulfing activity in astrocytes via alteration of astrocytic P2X7R.

2-YIA-31 YIA

A new mechanism for somatosensory information processing by descending noradrenergic pathway via spinal dorsal horn astrocytes

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The spinal dorsal horn (SDH) receives somatosensory inputs from the periphery and descending pain modulatory inputs from several brain regions including the locus coeruleus (LC). Recent progress has been made in understanding neuronal circuits in the SDH, but the role of astrocytes, one type of glial cells, in somatosensory information processing and behavior under physiological conditions is entirely unknown. Here, by establishing a method to monitor SDH astrocytic activities using an *in vivo* Ca^{2+} imaging technique, we revealed that superficial SDH astrocytes were activated following noxious stimulation by intraplantar capsaicin injection and that the astrocytic responses required activation of a_{1A}-adrenergic receptors (a_{1A}-AR) through descending noradrenergic signaling from the LC. Pharmacological inhibition of LC–SDH noradrenergic pathway and selective knockdown of a_{1A}-AR in superficial SDH astrocytes prevented capsaicin-induced pain hypersensitivity to light mechanical stimulation. Moreover, pharmacological activation of a_1-AR in superficial SDH astrocytes was sufficient to induce mechanical pain hypersensitivity. Our findings demonstrate for the first time the potential ability of superficial SDH astrocytes to modulate mechanosensory behavior as a non-neuronal gate for the descending noradrenergic pathway from the brain.

2-YIA-32 YIA

Involvement of glymphatic system in amyotrophic lateral sclerosis pathology

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Amyotrophic lateral sclerosis (ALS) is a motor neuron specific neurodegenerative disease. Accumulation of mutant Cu/Zn-superoxide dismutase (SOD1) protein aggregate in the spinal motor neurons is a common pathological hallmark in several types of ALS animal models and patients. The glymphatic system is a waste clearance system in the central nervous system: the directional flow of the cerebrospinal fluid (CSF) through the perivascular into interstitial spaces and the perivascular localization of aquaporin-4 (AQP4) promote its directional flow. Previously we reported that the AQP4 localization is aberrant and its expression is highly upregulated in SOD1-ALS mice during the progression of ALS symptoms (Watanabe et al., Neurosci Res, 133, 48-57, 2017). In the present study, we found the increase in the abnormal SOD1 protein deposition in SOD1-ALS/AQP4 knockout mice and the clearance of the protein from the spinal cord was slowed in AQP4 knockout mice. When we injected fluorescent labeled ovalbumin into the cisterna magna, the solute accumulation was greater in the SOD1-ALS mice than that in the wild-type mice. Our study suggests that the aberrant AQP4 distribution in the ALS model mice disrupts directional CSF flow and accelerates accumulation of toxic proteins in the spinal cord.

2-YIA-33 YIA

Anti-HMGB1 mAb therapy for intracerebral and subdural hemorrhage in rats

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High mobility group box-1 (HMGB1) is a ubiquitous and abundant nonhistone DNA-binding protein, and is also an important proinflammatory cytokine once released into extracellular space from the nuclei. In the present study, we examined the effects of anti-HMGB1 mAb on collagenase IV-induced intracerebral hemorrhage(ICH) and autologous blood-induced subdural hemorrhage(SDH) in rats. Here, we show that treatment with neutralizing anti-HMGB1 mAb (1mg/kg, twice) remarkably ameliorated ICH- and SDH- induced brain injuries. Administration of anti-HMGB1 mAb inhibited the release of HMGB1 into the extracellular space and reduced serum HMGB1 levels, thereby decreased the number of activated microglia and the expression of inflammation-related factors including TNF-α, iNOS, IL-1β, IL-6, IL-8R, COX-2 at 24h after ICH and TNF-α, iNOS, IL-1β at 48h after SDH. In chronic phase of ICH, we found that brain tissue loss and vasospasm were apparent, which was alleviated by the treatment of anti-HMGB1 mAb. Moreover, anti-HMGB1 mAb inhibited the body weight loss and improved the behavioral performance of rats. These results strongly indicate that HMGB1 plays a critical role in the development of ICH- and SDH- induced secondary injury through the amplification of plural inflammatory responses. Intravenous injection of neutralizing anti-HMGB1 mAb provides a novel therapeutic strategy for different types of stroke.

2-YIA-34 YIA

Aquaporin-4 facilitates paravascular space closure and neuronal activity reduction after water intoxication

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Rapid intraperitoneal water injection induces acute hyponatremia that creates an osmotic gradient driving for water entry into the brain, leading to subsequent cerebral edema. Paravascular spaces, which are covered by astrocyte end-feet, have been suggested to participate in the fluid circulation in cerebral cortex, however, it has not been clarified whether they morphologically change during the edema formation. Here we have established an *in vivo* imaging method with a closed cranial window under isoflurane anesthesia to observe paravascular spaces and astrocytes using CAG-GFP transgenic mice. We simultaneously monitored electro-corticogram (ECoG) and other physiological parameters, such as cerebral blood flow (CBF), heart rate, and arterial blood pressure, to examine their responses up to 40 min after the bolus injection of distilled water equal to 10% of body weight. We first confirmed that water injection indeed increased brain tissue water content, which was alleviated in aquaporin-4 (AQP4) knockout mice. While control and AQP4 knockout mice did not differ in the cell swelling of astrocyte, even AQP4 are expressed in the astrocyte end-feet, paravascular space closure was prevented in AQP4 knockout mice. Furthermore, the ECoG power reduction in AQP4 knockout mice was less than that in control mice. These results implicate that the regulation of paravascular spaces may play roles in modulating brain water circulation and brain edema formation, which might be controlled by AQP4.

2-YIA-35 YIA

The role of Prostaglandin D_2 synthase in retinal angiogenesis

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Although prostaglandin D_2 (PGD_2) represents anti-angiogenic role in tumor model, its role in physiological and pathological angiogenesis still remain unknown. We here evaluated the role of PGD_2 on retinal angiogenesis using genetically modified mice. In postnatal 8th day retina of WT, lipocalin-type PGD synthase (L-PGDS) was expressed in endothelial cells. Gene deficiency of L-PGDS impaired the physiological angiogenesis of retina, accompanied with increased mRNA expression of pro-angiogenic factor VEGF. *In vitro* study showed that L-PGDS inhibition elevated the hypoxia-induced VEGF expression, which was inhibited by treatment of a PGD_2 metabolite 15d-PGJ_2. We next generated a pericyte deficiency-induced retinal angiogenesis model by injection of anti-PDGFRβ antibody. In P8 retina of WT, the injection of antibody induces inflammation in retina, and infiltrating macrophages expressed hematopoietic PGD synthase (H-PGDS). Gene deficiency of H-PGDS or PGD receptor DP accelerated the angiogenesis. This phenomenon was accompanied with increased mRNA expression of one of the chemokines, Stromal derived factor 1α. In isolated macrophage, hypoxia increased the expression of cytokines, wich was inhibited by adding receptor inhibitor. Taken together, L-PGDS promotes physiological angiogenesis and H-PGDS attenuate pathological angiogenesis in mouse retina.

2-YIA-36 YIA

TRPC3-Nox2 complex formation mediates nutritional deficiency-induced cardiomyocyte atrophy

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Myocardial atrophy, characterized by the decreases in size and contractility of cardiomyocytes, is caused by severe malnutrition and/or mechanical unloading. Extracellular adenosine 5'-triphosphate (ATP), known as a danger signal, is recognized to negatively regulate cell volume. However, it is obscure whether extracellular ATP contributes to cardiomyocyte atrophy. Here, we report that ATP induces atrophy of neonatal rat cardiomyocytes (NRCMs) without cell death through P2Y2 receptors. ATP led to overproduction of reactive oxygen species (ROS) through increased amount of NADPH oxidase (Nox) 2 proteins, due to increased physical interaction between Nox2 and canonical transient receptor potential 3 (TRPC3). This ATP-mediated formation of TRPC3-Nox2 complex was also pathophysiologically involved in nutritional deficiency-induced NRCM atrophy. Strikingly, knockdown of either TRPC3 or Nox2 suppressed nutritional deficiency-induced ATP release, as well as ROS production and NRCM atrophy. Taken together, we propose that TRPC3-Nox2 axis, activated by extracellular ATP, is the key component that mediates nutritional deficiency-induced cardiomyocyte atrophy.

2-YIA-37 YIA

Heparan sulfate promotes the differentiation of muscle cells and contributes to maintain motor function in mice

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Heparan sulfate (HS) is a sulfated linear polysaccharide at the cell surface and in the extracellular matrix. HS plays an important role in various physiological and pathophysiological processes. Although previous studies showed the existence of HS in skeletal muscles, the roles of HS in these tissues remain unclear.

First, we examined the role of HS in the differentiation of muscle cells using C2C12 cells, a mouse myoblast cell line. CRISPR/CAS9 technology was used to delete Ext1, which encodes a heparan sulfate synthase. HS deletion dramatically impaired myoblast differentiation, demonstrating the essential role of HS *in vitro*. In order to confirm the importance of HS *in vivo*, we created skeletal muscle specific Ext1 knockout mice by Cre-loxP system (cKO). Muscle weakness of cKO was observed in treadmill tests and wire hang tests. Contraction of isolated soleus muscles from cKO was also impaired. Histological observation revealed that the cross sectional areas of various muscles were smaller in cKO. Electromicroscopic observation showed that myofibrils were thinner in cKO. Finally, we examined muscle differentiation after muscle injury by $BaCl_2$ injection to tibialis anterior muscle (TA). We showed the reduced expression level of myosin heavy chain and the increased number of centronucleated cells in cKO TA, indicating that the muscle regeneration after injury was attenuated in cKO.

These results demonstrate that HS plays an important role in skeletal muscle, especially in differentiation.

2-YIA-38 YIA

Characteristics of PDGFRα positive mesenchymal stromal cells in various tissues

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Mesenchymal stem cells are defined in vitro by the ability to form fibroblastic colony and differentiate into adipocytes, osteocytes, and chondrocytes. Although PDGFRα^+ cells are thought to be the origin of mesenchymal stem cells in various tissues, their roles in each organ have not been elucidated. Here, we compared characters of PDGFRα^+ cells derived from several organs such as lung, liver, small intestine, heart, subcutaneous fat, and skeletal muscle to clarify their specific functions in each organ.

We first compared differentiation potentials of PDGFRα^+ cells residing in various tissues. We cultured PDGFRα^+ cells isolated from each tissue by FACS and induced them to differentiate into several mesenchymal lineages. Consequently, each PDGFRα^+ population showed distinct differentiation potential. To investigate their roles in respective organs, we performed RNA-Seq and revealed that PDGFRα^+ cells have gene expression patterns unique to their original organ, suggesting that they have specific functions depending on the tissue where they reside. Among these tissues, we focused on skeletal muscle because PDGFRα^+ cells in muscle have been shown to be essential for homeostatic muscle maintenance. Using Using RNA-seq data of PDGFRα^+ cells from various tissues of young and aged mice, we identified several genes that are specifically expressed in PDGFRα^+ cells derived from young muscles. We expect that these genes play important roles to maintain muscle integrity and we will pursue a study to elucidate their functions.

2-YIA-39 YIA

Development of a novel functional assay to evaluate drug effects using human iPS cell-derived cardiomyocytes.

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Preclinical predictions using cell assay system is a major issue in drug development. With advances in iPS cell technology, human iPS cell-derived cardiomyocytes (hiPSC-CMs) are a valuable tool to characterize the pharmacological effects of drugs on heart cells. However, current approaches to evaluate cardiac contractile function *in vitro* are limited to low-throughput methods. We here test middle-through put and noninvasive assay system with motion field imaging (SI8000 system, Sony corporation) using high speed video image of hiPSC-CMs.

Human iPSC-CMs were kept at 37° C, 5% CO_2 and beating cells were recorded as sequential phase-contrast images. Motion vectors of hiPSC-CMs were analyzed by the SI8000 system. After the measurement, tissue-types (atrial or ventricular) were determined by immunostaining using anti-MLC2a and anti-MLC2v, respectively, and compared the motion vector traces. Contraction and relaxation velocities in atrial-like myocytes were faster than those in ventricular-like myocytes. Application of 100 nM isoproterenol induced the same trends on contractile functions in each cell-type of hiPSC-CMs, but beta2-antagonist blocked the effects only in atrial-like myocytes, indicating that the statistical comparison of these data allows us to identify tissue-types of hiPSC-CMs. Our results suggest a substantial potential to increase accuracy of pharmacological assessment.

2-YIA-40 YIA

Characterization of anti-atrial fibrillatory effect of anti-influenza drug oseltamivir assessed by the persistent atrial fibrillation dog, halothane-anesthetized dog and patch-clamp study

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Introduction: Anti-influenza drug oseltamivir delayed the atrial conduction and prolonged the atrial effective refractory period (AERP) in guinea pig hearts, and reduced the inducibility of burst pacing-induced atrial fibrillation (Af) in Langendorff-perfused rabbit hearts.

Methods: The canine persistent Af model (n=6) was prepared for the further in vivo characterization of the antiarrhythmic effect of oseltamivir. Moreover, we evaluated electropharmacological effect of oseltamivir on atria using the halothane-anesthetized dog (n=4). These results were compared with those of pure Na^+ channel blocker pilsicainide (n=6 and n=4, respectively). Furthermore, we evaluated the action of oseltamivir on ion channels expressed in HEK293 and CHO cells using the whole-cell patch-clamp technique (n=3).

Results: Oseltamivir (3 and 30 mg/kg) terminated the Af in 1 and 5 out of 6 animals, respectively, whereas pilsicainide (3 mg/kg) did it in 2 out of 6. Oseltamivir (0.3, 3 and 30 mg/kg) and pilsicainide (1 and 3 mg/kg) delayed the inter-atrial conduction in a dose- and frequency-dependent manner. Oseltamivir prolonged the AERP in a dose-dependent but frequency-independent manner, whereas pilsicainide did it in a dose- and frequency-dependent manner. IC_{50} values of oseltamivir against $I_{K,ACh}$, I_{Kr}, I_{Na}, I_{CaL} and I_{Kur} were 179, 225, >1000, >1000 and >1000 μM, respectively.

Conclusion: Oseltamivir can exert potent anti-Af effect through multi-channel inhibitory action, of which electrophysiological profile may be different from that of pilsicainide.

2-YIA-41 YIA

Effects of *Goreisan* on LPS-induced diarrhea and decrease in aquaporin 3 expression in intestinal epithelial cells.

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Goreisan is often used for gastrointestinal symptoms associated with bacterial and viral infections, to care diarrhea and to prevent general dehydration. Although several clinical reports have shown the effectiveness of *Goreisan*, pharmacological properties and underlining mechanism of *Goreisan* has not been clear. In this study, therefore, we investigated the antidiarrheic effect of *Goreisan* using a mouse model of enterocolitis induced by Lipopolysaccharide (LPS). *Goreisan* did not affect TNF-α mRNA expression, but markedly improved tissue injury and diarrhea scores. On the other hand, aquaporin-3 (AQP3) is expressed in the intestinal epithelium, and responsible for the absorption of water in the intestinal tract. Interestingly, both AQP3 mRNA and protein expression in the intestinal epithelium in LPS-treated group were significantly reduced, and *Goreisan* inhibited this decrease in AQP3. Decrease in AQP3 is thought to be associated with development of diarrhea, and therefore, *Goreisan* is estimated to have improved diarrhea symptoms by regulating the expression of AQP3. These results confirmed the effectiveness of *Goreisan* for infectious gastroenteritis, and it is also suggested a new effect of *Goreisan*.

2-YIA-42 YIA

Progranulin deficiency on macrophages exacerbates choroidal neovascularization *via* inflammation

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Chronic inflammation of the retina involves in the etiology of choroidal neovascularization (CNV), but the mechanisms are still not fully understood in detail. Progranulin is a growth factor secreted from myeloid cells and the deficiency of that results in aberrant inflammation in the central nerve system. The purpose of this study was to investigate the role of progranulin in the pathology of CNV.

By using grn knockout ($Grn^{-/-}$) and wild-type ($Grn^{+/+}$) mice with laser-induced CNV model, we evaluated the area of CNV and the accumulation of macrophages around CNV. To evaluate inflammation of macrophages, we constructed macrophage cell lines (RAW264.7) in which the expression of progranulin was knocked-down by RNA interference. Expression level of VEGF-A, IL-1β and C3 were evaluated by Western blotting.

At 14 days after laser injury, average of CNV area and number of Iba-1^{+} cells around CNV in the $Grn^{-/-}$ mice significantly increased compared with those in $Grn^{+/+}$. When progranulin was knocked down, the expression level of VEGF-A, IL-1β and C3 were increased in RAW264.7 cells.

These findings indicate that progranulin deficiency might promote the progression of CNV *via* aberrant activation of macrophages and microglial cells.

2-YIA-43 YIA

EP3 signaling in dendritic cells promotes liver repair after ischemia reperfusion injury in mice.

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Macrophage plasticity is essential for liver wound healing; however, the mechanisms of macrophage phenotype switch are largely unknown. Dendritic cells (DCs) are critical initiators of innate immune responses and orchestrate inflammation following hepatic injury. We have shown that PGE_2/EP3 promotes liver repair after hepatic ischemia-reperfusion (I/R). The present study examined whether signaling via EP3 in DCs regulates macrophage plasticity during liver repair by subjecting EP3-deficient ($EP3^{-/-}$) and wild-type (WT) mice to hepatic I/R. Compared with WT mice, $EP3^{-/-}$ mice showed delayed liver repair as indicated by increased levels of ALT and hepatic necrosis, which accompanied by reduced expression of hepatic growth factors. Flow cytometry analysis revealed that accumulation of $Ly6C^{low}$ reparative macrophages and monocyte-derived DCs (moDCs) was suppressed in $EP3^{-/-}$ livers. Adoptive transfer of moDCs from $EP3^{-/-}$ mice resulted in impaired repair, along with increased $Ly6C^{high}$ inflammatory macrophages. When bone marrow macrophages (BMMs) co-cultured with moDCs, BMMs from WT mice, but not from $EP3^{-/-}$ mice up-regulated expression of genes related to a reparative macrophage phenotype. In the presence of an EP3 agonist, interleukin (IL)-13 derived from moDCs drove BMMs to increase expression of genes characteristic of a reparative macrophage phenotype. The results suggest that EP3 signaling in moDCs facilitates liver repair by inducing IL-13-mediated switching of macrophage phenotype from pro-inflammatory to pro-reparative.

2-YIA-44 YIA

Prostaglandin $F_{2\alpha}$ receptor antagonist attenuated LPS-induced sepsis in mice.

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Sepsis is systemic inflammatory response syndrome caused by invasive infection. Although it is known that prostaglandin (PG)$F_{2\alpha}$ level is elevated in the plasma of the patients with sepsis, its role in the sepsis remains unclear. We aimed to investigate the role of $PGF_{2\alpha}$ receptor (FP) signaling in lipopolysaccharide (LPS)-induced sepsis using FP receptor antagonist AL8810 in mice. Sepsis was induced by intraperitoneal injection of LPS (5 mg/kg). AL8810 (10 mg/kg) was intraperitoneally administered at 30 min before LPS injection. Mice were monitored to detect the response to LPS for 24 hours. LPS administration promoted $PGF_{2\alpha}$ production in peritoneal lavage fluid (PLF). At 6 hours after LPS administration, the number of macrophages and neutrophils in PLF was increased, as compared with naïve mice. AL8810 administration enhanced neutrophil migration, but not macrophage migration, in PLF. At 24 hours after injection, there was no difference in number of these cells between LPS and/or AL8810-administered mice. At 24 hours after LPS administration, the mRNA expression of proinflammatory cytokines such as IL-6, TNF-α, IL-1β, and CXCL2 in lung and liver was elevated. Conversely, they were decreased in AL8810-administered mice. It is known that IL-10 decreased excessive inflammatory responses in the acute phase of sepsis. At 3-6 hours after LPS administration, IL-10 levels in PLF were increased, as compared with naïve mice. AL8810 administration enhanced IL-10 production further. In addition, immunostaining showed that Gr-1-positive neutrophils in PLF expressed IL-10. Then, anti-IL-10 antibody administration increased LPS-induced IL-6 and CXCL-2 expression as well as AL8810-decreased these gene expressions. The findings suggest that FP receptor antagonist attenuated LPS-induced sepsis by increasing neutrophil-derived anti-inflammatory cytokine IL-10 production.

2-YIA-45 YIA

Caveolin-1 regulates P2X7-mediated ATP signaling in pro-inflammatory macrophages.

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[Background] Macrophage (Mϕ) plays crucial roles in innate immunity and its dysfunction is involved in the pathogenesis of chronic inflammatory diseases such as arteriosclerosis and diabetes. Cytokine secretion and phagocytosis are main functions of Mϕ and modulated by the activity of ion channel, ionotropic purinergic P2X7 receptor.

Caveolin-1 (Cav-1) enables effective intracellular Ca^{2+} signaling by accumulating Ca^{2+} channels and their associated proteins within caveolae structure. In this study, the functional coupling between Cav-1 and P2X7 receptor was analyzed using Cav-1 knockout (Cav-1 KO) mice.

[Methods] In murine bone marrow-derived Mϕ (BMDM), expression of Cav-1 was analyzed by real-time PCR and Western Blotting. Localization of Cav-1 and P2X7 receptor was analyzed with total internal reflection fluorescence microscope (TIRFM). Ca^{2+} influx and K^+ efflux through P2X7 receptor were measured with Fluo-4 AM and APG-2, respectively. Furthermore, activation of P2X7 receptor was measured by nuclear dye (TOPRO-3) uptake.

[Results] The expression of Cav-1 was increased by LPS (lipopolysaccharide, 1 μg/mL)-induced inflammatory stimulation in BMDM. Thereafter, Cav-1 was co-localized with P2X7 receptor on the cell membrane. ATP (1 mM)-evoked TOPRO-3 uptake was increased in BMDM derived from Cav-1 KO mice compared to WT. Furthermore, Ca^{2+} influx and K^+ efflux following ATP stimulation were increased in Cav-1 KO compared to WT. These results suggest that the activity of P2X7 receptor is enhanced and thus Ca^{2+} influx and K^+ efflux are facilitated in BMDM derived from Cav-1 KO mice.

[Conclusion] Cav-1 negatively regulates the activation of P2X7 receptor and modulates immune responses in Mϕ. This study may contribute to the development of novel drugs for chronic inflammatory diseases.

2-YIA-47 YIA

Regulatory Mechanisms of Primary Ciliary Resorption and Cell Cycle Progression by a Dynein Light Chain, Tctex-1

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The primary cilium is a microtubule-based sensory organelle that transduces its signals through specifically distributed receptors and ion channels on the ciliary membrane. The proximal region of the ciliary axoneme is surrounded by an invaginated membrane, called ciliary pocket. Primary cilium is formed during the G0/G1 phase in many cell types, including neural progenitor cells, and is resorbed as the cells re-enter cell cycle. Dysregulation of the ciliary dynamics is associated with hereditary disorders, such as microcephaly. Tctex-1, a cytoplasmic dynein light chain, has a dynein-independent role when it is phosphorylated at Thr94. We have shown that (T94)Tctex-1 phosphorylated by the action of insulin-like growth factor 1 accelerates branched actin organization and clathrin-dependent endocytosis at the ciliary pocket. The machinery was critical for ciliary resorption, cell cycle re-entry, and self-renewal of the neural progenitor cells in the developing neocortex. However, it remains unclear how Tctex-1 regulates the endocytosis. In the present study, we identified microtubule-associated serine/threonine kinase 4 (MAST4), a function-unknown protein, as a binding protein to Tctex-1. In retinal pigmented epithelial cells (RPE-1), a model cell line for cilia researches, we found that knockdown of MAST4 suppressed endocytosis, ciliary resorption, and cell cycle re-entry, emphasizing on the significance of phospho-(T94)Tctex-1-MAST4 pathway as a part of such biological events.

2-YIA-48 YIA

Identification of a chemical chaperone for preventing protein aggregation and proteotoxicity under endoplasmic reticulum stress

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Endoplasmic reticulum (ER) is responsible for protein biosynthesis and folding, but accumulation of unfolded proteins leads to disturbance of ER proteostatis and subsequent clinical pathologies including diabetes, neurodegenerative disease and cancer. Chemical chaperones are chemical compounds that help protein folding and suppress aggregation, and receiving increased attention as potential therapeutic approaches for ER stress-related diseases. In this study, we established a novel ER stress reporter cell line and identified compound X as a chemical chaperone from the 217,765-compound chemical library. Compound X directly binds to secreted or membrane proteins and inhibits protein aggregation during tunicamycin induced ER stress. Furthermore, compound X significantly prevented cell death caused by chemically induced ER stress and by an aggression-prone mutant prion protein. These results show the therapeutic potential of compound X as a chemical chaperone that can ameliorate ER stress-related diseases.

2-YIA-49 YIA

Lysosomal Regulation of mTOR-AKT Signaling via the Vacuolar-type H^+-ATPase

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Vacuolar-type H^+- ATPase (V-ATPase), a multi-subunit protein complex, has two distinct functions on lysosomes: acidifying the lysosomal lumen and controlling mTOR-S6K (mTORC1) signaling via Ragulator. Both functions are crucial for several biological processes. However, little is known about how the functions are coordinated and whether V-ATPase also regulates mTOR-AKT (mTORC2) signaling. We found that knocking down (KD) of a subunit of V-ATPase in human induced pluripotent stem cells (hiPSCs) impaired its functions: increasing lysosomal pH and decreasing mTORC1 signaling. Unexpectedly, the KD also attenuated mTORC2-AKT signaling. Treatment of hiPSCs with bafilomycin A1, a specific inhibitor of V-ATPase proton pump activity, increased lysosomal pH as expected, and decreased both mTORC1 and mTORC2 signaling activities. Therefore, in addition to mTORC1, V-ATPase seemingly regulates the mTORC2-AKT. We are now investigating how V-ATPase regulates mTORC2. Furthermore, we are examining the effects of V-ATPase inhibition on the mTOR signaling *in vivo*. We will discuss our results in this meeting.

2-YIA-50 YIA

ATP increases ciliary beat frequency in mouse airway cilia through $P2Y_1$ receptor

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Mucociliary transport, which is a host-defense mechanism of the airway, consists of the mucous layer and the beating cilia lining on the airway surface. Although beating of cilia is the most important in this system, the regulation of beating is not fully understood. Among a few pharmacological stimuli which has been known to increase the ciliary beating, ATP is one of the most effective. However, ATP is not useful expectorant, because of its wide-spread pharmacological activity. In the present study, therefore, we have examined the purinergic receptor, which is involved in the increase in ciliary beating by ATP, in isolated mouse airway cilia. ATP significantly increased both ciliary beat frequency (CBF) and ciliary bend angle (CBA), whereas ADP increased only CBF. In contrast, adenosine and UTP did not increase CBF and CBA. Interestingly, increase in CBF by ATP was abolished by BAPTA-AM, but CBA was not affected, suggesting that ATP differently regulates CBF and CBA. Finally, increase in CBF by ATP was completely inhibited by MRS2179, a $P2Y_1$ receptor antagonist. Therefore, we may propose $P2Y_1$ receptor agonist as a new airway clearance stimulator, which increases ciliary beating.

2-YIA-51 YIA

Activation of dopamine D1 receptor-expressing medium spiny neurons in the nucleus accumbens directly suppresses the tumor progression

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2-YIA-52 YIA

Morphological changes in striatum and nucleus accumbens neurons lead to abnormal behavior in ARHGAP10 mutant mice

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Schizophrenia is a severe mental illness that affects about 1% of the population. Genetic and environmental factors contribute to the development of schizophrenia. However, the exact pathoetiology remains unclear. We generated Rho GTPase-activating protein 10 (ARHGAP10) mutant mice carrying similar variations found in Japanese schizophrenia patients. In the present study, we examined spatiotemporal expression of ARHGAP10 mRNA in the brain of mice. The expression levels of ARHGAP10 mRNA were higher in the striatum (ST) and nucleus accumbens (NAc) than those in other brain regions. We performed a series of behavior test to evaluate cognitive and emotional function in ARHGAP10 mutant mice. They showed an increase in anxiety level, and manifested potentiation of methamphetamine-induced hyperlocomotion and visual discrimination task. Morphological analysis revealed that methamphetamine-treated ARHGAP10 mutant mice showed an increase in the number of c-Fos-positive-cells in the dorsal medial striatum (dmST) and NAc core than those in wild-type littermates. Golgi staining indicated that ARHGAP10 mutant mice showed an increase in neuronal complexity and spine density in the same brain regions compared to the wild-type mice. These results suggest that ARHGAP10 gene variations may lead to the development of cognitive and emotional deficits with morphological abnormality in the dmST and NAc core neurons.

2-YIA-54 YIA

Hypothalamic dopaminergic functions negatively regulate feeding behavior

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Role of central nervous systems in regulation of energy homeostasis including feeding behavior has paid much attention these days, but their mechanisms are still unclear. Since the hypothalamus is a key regulator in feeding behavior, we investigated the role of dopaminergic functions in the lateral hypothalamus (LH) in feeding behavior. Both food intake and glucose injection increased dopamine levels in the LH. When retrograde tracer Fluoro-Gold (FG) was injected to the LH, the FG-positive cells were present in the ventral tegmental area (VTA) and the substantia nigra pars compacta (SNC), which were tyrosine hydroxylase-positive. Injections of both dopamine D_1 (SKF 38393) and D_2 (quinpirole) receptor agonists into the LH decreased food intake, which were antagonized by respective antagonist. When the dopaminergic activity in the LH was inhibited by a Ca^{2+} channel inhibitor pregabalin, pregabalin inhibited the increase of dopamine levels induced by glucose injection, and it also increased food intake. These results have indicated that food intake activates dopamine neurons projecting from the VTA and the SNC to the LH through increase in the blood glucose levels. Moreover, it is suggested that the promotion of dopaminergic functions in the LH terminates feeding behavior by the stimulation of dopamine D_1 and D_2 receptors.

2-YIA-55 YIA

Involvement of GPR143 in the hippocampal pathophysiological alteration after limbic seizures

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Temporal lobe epilepsy (TLE) is the most common form of epilepsy. The hippocampus, located in the mesial temporal lobe, is implicated in the development of TLE. However, mechanisms underlying hippocampal epileptogenesis in TLE remain unclear. Here, we investigated whether ocular albinism 1 gene product (GPR143), which is highly expressed in the hippocampus, is involved in hippocampal epileptogenesis in TLE. We induced limbic seizures by administration of kainic acid. We found that seizure scores reduced in *Gpr143*-gene deficient (GPR143-KO) mice compared to *wild-type* (*wt*) mice. Next, we performed histological examination. To evaluate granule cell reorganization, we measured the width of the granule cell layer 6 days after seizure induction. The granule cell layer dispersed less in GPR143-KO mice than *wt* mice. We further found that an increased number of survival neurons and a morphological change of microglia in the CA3 region and its surrounding area in GPR143-KO mice, respectively. Thirty days after seizure induction, we observed aberrant sprouting of granule cell axons in the molecular layer. We immunohistochemically assessed the distribution of synaptoporin, a protein that is often present in the mossy fiber boutons, in the molecular layer. The intensity of ectopic synaptoporin signals decreased in GPR143-KO mice, suggesting that mossy fiber sprouting occured less compared to *wt* mice. Thus, our findings indicate that GPR143 is involved in the modulation of seizure phenotype and hippocampal epileptogenesis in TLE.

2-YIA-56 YIA

Functional Evaluation of Neutrophils Spheroidized by HRG

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[Background]
Histidine-rich glycoprotein (HRG) is 75 kDa plasma glycoprotein produced from the liver. In previous study, we reported that HRG treatment prevents lethality of sepsis model mice and HRG regulated spherical shape change, passage of microcapillary and production of extracellular ROS on the human neutrophils. Next, we analyzed functional evaluation of neutrophils spheroidized by HRG.
[Method]
Phagocytosis analysis: We quantified the area of fluorescence-labeled bacteria by pHrodo in the neutrophils. Viability analysis: The number of intact neutrophils were counted by the staining with calcein-AM. Determination of extracellular ROS production: After adding isoluminol, HRP and each test reagent to neutrophils, the intensity of luminescence at 30 minutes were measured.
[Result]
Neutrophils treated with HRG showed increased activity of phagocytosis in a dose-dependent manner. HRG also induced high survival rate. When Zymosan A was added to neutrophils, the increased ROS production was observed in the presence of HRG.
[Discussion]
The neutrophils treated with HBSS or HSA are firmly attached to the bottom of the plate and being stimulated with regard to ROS production. In contrast, HRG maintained the spherical shape of neutrophils, phagocytic activity and responsiveness to Zymosan A. These results suggested that HRG may act on neutrophils to suppress excessive adhesion to vascular endothelium under normal condition and induce the functional activation when neutrophils meet bacteria.

2-YIA-57 YIA

VEGFR1 signaling plays a critical role in endometriosis through increasing lymphangiogenesis

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Lymphangiogenesis is associated with the growth of endometriosis. In this study, we examined the role of vascular endothelial growth factor (VEGF) receptor 1 (VEGFR1) signaling in lymphangiogenesis and tissue growth in an endometriosis model. Using wild-type (WT) and VEGFR1 tyrosine kinase (TK) deficient mice, endometrial fragments were implanted into the peritoneal wall of mice. Endometrial tissue growth and lymphangiogenesis as indicated by lymphatic vessel density were determined. Endometrial fragments from wild-type (WT) mice transplanted into in host WT mice (WT→WT) grew with increased lymphangiogenesis accompanied by increases in pro-lymphangiogenic factors, VEGF-C and VEGF-D. The implant size and lymphangiogenesis were reduced in the $TK^{-/-}$→$TK^{-/-}$. Immunofluorescence demonstrated that both VEGF-C and VEGF-D were expressed in both $CD11b^{+}$ and $S100A4^{+}$ cells. When cultured bone marrow-derived macrophages and fibroblasts were stimulated with placental growth factor (PlGF), a specific agonist for VEGFR1, the mRNA levels of VEGF-C and VEGF-D were increased in a VEGFR1 dependent manner. A VEGFR3 kinase inhibitor significantly suppressed the size of implants, lymphangiogenesis, pro-lymphangiogenic factors, and accumulation of $CD11b^{+}$ and $S100A4^{+}$ cells. These results suggest that VEGFR1 signaling in macrophages and fibroblasts promote the growth and lymphangiogenesis in endometrial tissue. Therefore, VEGFR1 blockade is a potential treatment for endometriosis.

2-YIA-58 YIA

TP signaling in immune cells promotes lymphangiogenesis in the diaphragm during peritonitis

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Lymphangiogenesis has functional consequences not only for lymphatic transport, but also for inflammation resolution. Thromboxane A_2 (TxA_2) has been suggested to involve not only in induction of inflammation, but also in resolution of inflammation. We investigated the functional role of TxA_2 receptor (TP) signaling in inflammation-associated formation of newly lymphatic vessels. Lymphangiogenesis in the diaphragm of TP knockout mice (TPKO) or their wild-type (WT) counterparts was induced by repeated intraperitoneal injection of LPS. Compared with WT, LPS-induced lymphangiogenesis in TPKO was suppressed, which was accompanied by reduced expression of vascular endothelial growth factor (VEGF)-C and VEGF-D in CD11b+ and CD4+ cells in diaphragm tissue. TP was expressed in CD11b+ and CD4+ cells, but not in LYVE-1+ cells (lymphatic vessels). U46619, an agonist for TxA_2, did not proliferate cultured lymphatic endothelial cells. As compared with controls, mice with macrophage TP receptor deletion showed attenuation of lymphangiogenesis with reduced expression of VEGF-C and VEGF-D. When fluorescein isothiocyanate (FITC)-dextran was injected into the peritoneal cavity, the amount of residual FITC-dextran in macrophage-specific deletion of TP receptor was greater than that in controls. The same was true for mice with T cell TP receptor deletion. The present results suggest that TP signaling in macrophages and T cells plays a critical role in inflammation-related lymphangiogenesis and drainage function of lymphatics in the diaphragm.

2-YIA-59 YIA

RAMP1 signaling facilitates angiogenesis and lymphangiogenesis in the endometriotic lesions in mice

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Newly formation of blood and lymphatic vessels is involved in the development of endometriosis. We have demonstrated that calcitonin gene-related peptide (CGRP) promotes wound healing and wound-associated formation of blood and lymphatic vessels via receptor activity-modifying protein 1 (RAMP1), a subunit of the CGRP receptor. In the present study, using wild-type (WT) mice and RAMP1 deficient ($RAMP1^{-/-}$) mice, we examined whether RAMP1 plays a role in the growth of endometriosis by angiogenic responses. Ectopic endometriosis model was created by transplantation of endometrial tissue fragments from donor mice into the peritoneal wall of host mice. The sizes and density of blood and lymphatic vessels in the $RAMP1^{-/-}$ implants from host $RAMP1^{-/-}$ mice ($RAMP1^{-/-}$ →$RAMP1^{-/-}$) were reduced as compared with the WT→WT. The mRNA levels of markers for blood and lymphatic vessels as well as growth factors for angiogenesis and lymphangiogenesis in the $RAMP1^{-/-}$→$RAMP1^{-/-}$ were lower than those in the WT→WT. Immunofluorescence demonstrated that RAMP1 was expressed in $CD11b^{+}$ and $S100A4^{+}$ cells, and these cells also co-localized with VEGF-A, VEGF-C, and VEGF-D. Cultured macrophages and fibroblasts increased the mRNA levels of VEGF-A, VEGF-C, and VEGF-D in a RAMP1 dependent manner. These results suggest that RAMP1 signaling in macrophages and fibroblasts is critical for the growth of endometriosis by promoting angiogenesis and lymphangiogenesis.