1-YIA-01

Genetical and pharmacological inhibition of ATP-sensitive potassium channels induces anxiety-like behavior in mice

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Although Kir6.2, one of the subunits composed ATP-sensitive potassium (K_{ATP}) channels, are widely distributed in the brain, the roles in emotional behavior are not yet fully understood. Here we investigated the behavioral characteristics of Kir6.2-deficient ($Kir6.2^{-/-}$) mice. $Kir6.2^{-/-}$ mice showed anxiety-like behavior in the elevated-plus maze test and light-dark test, especially, it was prominent in females. Immunohistochemical studies showed that Kir6.2 was co-localized with tryptophan hydroxylase (TPH) in the dorsal raphe nuclei and tyrosine hydroxylase (TH) in the ventral tegmental area/locus coeruleus. Interestingly, TPH expression in the midbrain was significantly elevated in female $Kir6.2^{-/-}$ mice. These results suggest that Kir6.2 expressed in serotonergic neurons could play a key role in emotional behavior. Furthermore, we investigated whether pharmacological blockade of K_{ATP} channels affects the emotional behavior. Mice that had been injected intracerebroventricularly with glibenclamide, a selective K_{ATP} channel blocker, showed anxiety-like behavior in the elevated-plus maze test. These results confirm a critical role of K_{ATP} channels in regulation of emotional behavior.

1-YIA-02

The development of novel anti-depressant targetting AMPA receptor

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Depression is the major mental disorder and over one million patients are suffering from this disease. It was also reported that the number of patients showing resistance toward anti-depressant, i.g. SSRI and SNRI, got increase. We have already known that molecular mechanism underlying depression is heterogeneous so that it is hard to estimate the efficacy of anti-depressant without molecular rationale. Postmortem human brain analysis indicated that the number of AMPA receptors (AMPARs), major molecule controlling synaptic functions, varied among depression patients and the results of these analysis were not consistent. To clarify the dynamics of AMPARs in depression patients, we developed the novel PET imaging method to measure the density of AMPARs in depression patients. This result showed that depression patients decreased AMPARs broadly throughout the brain. This fact motivated us to develop novel AMPARs potentiator in order to cure the depression. To find the compound showing high affinity to AMPARs and high BBB penetratability, we modified the compound named PEPA, already known to bind specifically to AMPARs, and finally succeeded in synthesizing the seed compound. This compound could exert the anti-depressant effect quickly and sustained for a week after the cessation of drug administration. Furthermore, this anti-depressant effect was significantly stronger that another AMPARs potentiators.

1-YIA-03

Effects of desmoplakin knockdown in the hippocampus on lipopolysaccharide (LPS)-induced depressive-like behaviors

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Desmosome is a cell structure for cell-to-cell adhesion in epithelia and cardiac muscles. Desmoplakin, a component of desmosome, is highly expressed in the dentate gyrus (DG) in the brain, although there is no evidence for desmosome structures in neurons. We previously found that the expression of desmoplakin in mouse hippocampus is decreased by antidepressant treatments, including selective serotonin reuptake inhibitors (SSRIs) and electroconvulsive stimulation (ECS), a model of electroconvulsive therapy. However, the role of desmoplakin in depressive-like behaviors has been unknown. In this study, we examined the contribution of knockdown of desmoplakin to depressive-like behaviors. To knockdown desmoplakin, we generated adeno associated virus expressing artificial microRNA targeting desmoplakin and injected it into the mouse DG. LPS was intraperitoneally administered, and sucrose preference and forced swim tests were used for assessing depressive-like behaviors. Knockdown of desmoplakin in the DG partly attenuated LPS-induced depressive-like behaviors. These results implicate that down-regulation of desmoplakin expression by antidepressant treatment may be involved in antidepressant effects in the hippocampus.

1-YIA-04

Depletion of microglia ameliorates cognitive impairment in a mouse chronic cerebral hypoperfusion model

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Chronic cerebral hypoperfusion (CCH) is manifested in a various CNS diseases, including neurodegenerative and mental disorders accompanied by cognitive impairment. We previously demonstrated that microglial activation via TRPM2, a Ca^{2+}-permeable non-selective cation channel, 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), while there was no direct evidence on the contribution of microglia. To clarify the role of microglia in the BCAS model, we used PLX3397, an orally-active inhibitor of colony-stimulating factor 1 receptor (CSF1R), since microglia require CSF1R signaling for survival. When mice were fed with PLX3397-containing chow at 290 mg/kg for 3 weeks, virtually all Iba1-immunopositive microglia were eliminated from the brains, without obvious deficits in the behaviors. When the mice were then subjected to BCAS, white matter injury and cognitive dysfunction at additional 28 days were improved in the PLX3397-fed mice compared with control mice. These results suggest that microglia play destructive roles in the development of CCH-induced cognitive impairment.

1-YIA-05

Spinal dorsal horn astrogliosis facilitates itch transmission in a mouse model of contact dermatitis.

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Chronic itch is a major symptom in various skin diseases, such as atopic and contact dermatitis, but its mechanism remains to be determined. We have previously shown that spinal dorsal horn (SDH) astrocytes become activated in mouse models of chronic itch and that astrocyte-derived lipocalin-2 (LCN2) is crucial for maintaining chronic itching. However, how LCN2 enhances spinal itch neurotransmission is not understood. In this study, using *Gastrin-releasing peptide receptor (Grpr)-egfp* mice to label itch-specific neurons in SDH, we found GRP-induced depolarization of excitatory $GRPR^+$ neurons was greatly potentiated in contact dermatitis model mice. Genetic inhibition of signal transducer and activator transcription 3 (STAT3) in SDH astrocytes ameliorated chronic itch and also normalized enhancement of GRP-induced depolarization of excitatory $GRPR^+$ neurons in chronic itch model. Furthermore, coadministration of LCN2 and GRP also potentiated GRP-induced depolarization of excitatory $GRPR^+$ neurons. Our finding indicate that reactive astrocytes facilitated SDH itch transmission in chronic itch via upregulated LCN2.

1-YIA-06

Histidine-rich glycoprotein inhibits high mobility group box 1-mediated signal pathway in vascular endothelial cells

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A pathogenic role for high-mobility group box 1 (HMGB1) protein has been postulated in severe sepsis. Histidine-rich glycoprotein (HRG) is a 75-kDa plasma protein which was recently proposed as a new biomarker to predict the outcome of sepsis patient and was demonstrated to improve the survival of septic mice through the regulation of neutrophils and vascular endothelial cells. Here, we monitored the effects of HRG on the lipopolysaccharide (LPS)-mediated release of HMGB1 and the HMGB1-mediated modulation of proinflammatory responses in EA.hy 926 endothelial cells. Our results show that LPS induced significant HMGB1 translocation from nucleus to cytoplasma and large amount of HMGB1 release from EA. hy 926 endothelial cells which effectively inhibited by HRG. Furthermore, HRG potently inhibited high expression of adhesion molecules and release of inflammatory cytokines from HMGB1-activated endothelial cells. HMGB1 up-regulated proinflammatory responses by interacting with three pathogen-related pattern recognition receptors: TLR2 and TLR4 and RAGE. HRG also down-regulated the cell surface expression of all three HMGB1 receptors in endothelial cells. The protective effects of HRG in severe sepsis may partially be mediated through the inhibition of HMGB1 translocation/release.

1-YIA-07

The beneficial effect of STAT3 decoy oligodeoxynucleotide transfection on multiple organ injury in mice with cecal ligation and puncture-induced sepsis

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Sepsis is regarded as a gene-related disorder. Growing evidence suggests that STAT3 is a master transcriptional factor that plays an important role in inflammation. In this study, we examined whether in vivo introduction of STAT3 decoy oligodeoxynucleotides (ODNs) can provide benefits in mice with cecal ligation and puncture (CLP)-induced sepsis to assess the potential role of STAT3 in sepsis-associated organ dysfunction. Activation of STAT3 greatly increased in each of major organs after CLP in time dependent manner. We confirmed that STAT3 decoy ODNs were effectively delivered into tissues of septic mice in vivo by preparing into a complex with atelocollagen given 1 h after CLP. When STAT3 decoy ODNs were given to septic mice, abnormal production of pro-inflammatory and chemotactic cytokines was significantly reduced, histopathologic changes in lung, liver, and kidney tissues were markedly improved, and led to a significant survival advantage in mice after CLP. The STAT3 inhibitor stattic mimicked the beneficial effects of STAT3 decoy ODN transfection in septic mice. These results suggest that STAT3 is a potential therapeutic target for sepsis-associated multiple organ injury.

1-YIA-08

Myeloid-derived suppressor cells (MDSCs) are involved in anti-asthmatic effect of glucocorticoid in OVA-sensitized mice

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Myeloid-derived suppressor cells (MDSCs) are heterogeneous population of immunosuppressive myeloid progenitor cells. We have previously shown that MDSCs are increased and play protective role in OVA-sensitized mice lung. On the other hand, glucocorticoid is the most commonly used anti-inflammatory drug for asthma. The aim of this study is, therefore, to define the potential role of MDSCs in anti-asthmatic effect of glucocorticoid. Dexamethasone (DEX) enhanced differentiation from bone marrow cells into MDSCs under co-stimulation of IL-6/GM-CSF *in vitro*, which was inhibited by mifepristone, a glucocorticoid receptor antagonist. DEX-treated MDSCs showed potent inhibitory effect of T cell proliferation. Consistent with *in vitro* results, administration of DEX significantly increased a population of MDSCs, accompanied by resolution of inflammation in lung of OVA-sensitized mice. By contrast, inhibition of MDSCs attenuated the effect of DEX. Taken together, these data reveal a novel role of MDSCs in anti-inflammatory effect of glucocorticoid and further implicated pharmacological targeting of MDSCs as a potential therapeutic strategy in asthma.

1-YIA-09

RAMP1 signaling in immune cells regulates lymphangiogenesis in the diaphragm during peritonitis

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Lymphatic vessels in the diaphragm are essential for draining peritoneal fluid during peritonitis. Calcitonin gene-related peptide (CGRP), which is released from the sensory nervous system, promotes wound-induced lymphangiogenesis via receptor activity-modifying protein 1 (RAMP1), a subunit of the CGRP receptor. In this study, we examined the functional role of RAMP1 in inflammation-induced lymphangiogenesis in the diaphragm. RAMP1-knockout mice (RAMP1 KO) or their wild-type counterparts (WT) were intraperitoneally injected with LPS. Compared with WT, RAMP1 KO exhibited less lymphangiogenesis associated with reduced expression of VEGF-C and VEGF-D and with enhanced expression of Th1-related cytokines including TNF and IFN. The numbers of CD4+ cells in WT were greater than those in RAMP1 KO. RAMP1 was expressed in CD4+ cells, which also expressed VEGF-C and VEGF-D. Isolated splenic CD4+ cells stimulated with LPS enhanced expression of VEGF-C and VEGF-D in a RAMP-1 dependent manner. Deletion of CD4+ cells with an anti-CD4 antibody suppressed lymphangiogenesis. Functional assays with an intraperitoneal injection of fluorescein isothiocyanate (FITC) revealed delayed peritoneal fluid drainage in diaphragm of WT as compared with RAMP1 KO. These results suggest that RAMP1 signaling in T cells plays a critical role in LPS-induced lymphangiogenesis and lymphatic dysfunction in the diaphragm.

1-YIA-10

Regulation of mRNA translation machinery in influenza virus infection

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Virus infection is generally associated with virus-driven hijacking of host cellular machineries including translational regulation. Host shutoff is known as a strategy used by viruses to repress cellular mRNA translation and parallelly allow the efficient translation of viral mRNA. Host shutoff could be achieved by two complementary mechanisms: either direct co-opting of the translation machinery that forces better translation of viral mRNAs compared to their host counterparts, or viral-induced degradation of host mRNAs. However, it remains unclear how host shutoff and viral mRNA translation is regulated upon influenza virus infection. In the present study, to systemically analyze the dynamic changes in host and viral mRNA translation machinery, we performed ribosome profiling and RNA sequencing (RNA-seq) analysis in the mouse embryonic fibroblasts infected with influenza A (H1N1/PR8) virus. We will report the global profiles and characteristics of gene expression and translational states for host and virus mRNA during the course of influenza infection, which could lead to a better understanding of mRNA translation machinery in influenza virus infection.

1-YIA-11

Distinct synaptic mechanisms may underlie the analgesic effects of GAT1 and GAT3 inhibitors

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Since a sustained higher excitability in the superficial dorsal horn is considered to be a crucial factor leading to chronic pain, normalizing excitatory and inhibitory balance in the dorsal horn by inhibiting GABA transporters (GAT1 and GAT3) is a promising therapeutic strategy for pain relief. However, synaptic mechanisms underlying the analgesic effects of GAT inhibitors remain unknown. Using spinal slice preparations from adult mice, we previously demonstrated that the GAT1 inhibitor NNC -711 decreases the frequency of miniature EPSCs (mEPSCs) in the dorsal horn neurons via presynaptic mechanisms including the activation of $GABA_B$ receptors. In the present study focusing on GAT3 inhibition, we found that the GAT3 inhibitor SNAP-5114 suppressed monosynaptic C-fiber-evoked EPSCs, which was not observed in the presence of the $GABA_B$ receptor antagonist CGP55845. By contrast, A-fiber-evoked EPSCs were less suppressed, and the frequency and the amplitude of mEPSCs were not altered. Thus, although endogenously increased GABA after blockade of GAT1 and GAT3 acts on $GABA_B$ receptors, GAT1 and GAT3 inhibition depresses excitatory neurotransmission from spinal intrinsic interneurons and the primary afferent fibers, respectively.

1-YIA-12

Reappearance of astrocytic mGluR5 assembles cortical networks in induction of neuropathic pain

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Astrocytes play essential roles for the modulation of neural networks, but also have critical roles for the pathogenesis of neural disorders. Recently, we revealed that astrocytes in the primary somatosensory cortex (S1) have a critical role for neuropathic pain after partial sciatic nerve ligation (PSNL). In brief, PSNL generated Ca^{2+} excitation in S1 astrocytes, induced synaptogenesis, thereby resulting in cross-wiring of innocuous and nocuous circuits. Reappearance of mGluR5 correlated with neuropathic pain, but we still do not know whether astrocytic mGluR5 is required or not. Here, we show that mGluR5 in S1 astrocytes is a cause of cortical rewiring and neuropathic pain. Firstly, we found that mGluR5 is almost absent but reappeared in S1 astrocytes after PSNL. Secondly, we made brain astrocyte-specific mGluR5 knockout mice (astro-mGluR5-KO) and validated them. PSNL-induced mechanical allodynia was abolished in astro-mGluR5-KO mice, suggesting that upregulation of mGluR5 in S1 astrocytes should be required for mechanical allodynia. Third, mechanisms underlying astrocytic mGluR5-mediated allodynia were; (1) increase in Ca^{2+} activity, (2) expression of synaptogenic molecules such as glypican4 and hevin, (3) synaptogenesis, (4) persistent rewiring of incorrect S1 circuits. Hence, we conclude that astrocytic mGluR5 is a crucial molecule that triggers synaptogenesis in S1 after PSNL, which is the causative event for the pathogenesis of neuropathic pain.

1-YIA-13

New pharmacological effect of fulvestrant to prevent oxaliplatin-induced peripheral neuropathy

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The anti-cancer drug oxaliplatin frequently causes peripheral neuropathy. Commonly described neuropathic symptoms include aberrant sensations such as mechanical allodynia (hypersensitivity to normally innocuous stimuli). Although oxaliplatin neuropathy is a dose-limiting toxicity, preventive strategies against its side effects have not been established. We screened several sets of small-molecule chemical libraries (more than 3,000 compounds in total) using a newly established *in vitro* high-throughput phenotypic assay, and identified fulvestrant, a clinically approved drug for the treatment of breast cancer in postmenopausal women, as having a protective effect on oxaliplatin-induced neuronal damage. Furthermore, using a rat model of oxaliplatin neuropathy, we demonstrated the *in vivo* efficacy of fulvestrant to prevent oxaliplatin-induced axonal degeneration of the sciatic nerve and mechanical allodynia in histological and behavioural analyses. Thus, our findings reveal a previously unrecognised pharmacological effect of fulvestrant to prevent oxaliplatin-induced painful peripheral neuropathy and may represent a novel prophylactic option for patients receiving oxaliplatin chemotherapy.

1-YIA-14

Hepatic disorder is a risk factor for aggravation of oxaliplatin-induced peripheral neuropathy in humans and mice: Possible involvement of HMGB1

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We have shown a crucial role of HMGB1 in chemotherapy-induced peripheral neuropathy (CIPN). To clarify risk factors for CIPN, we retrospectively analyzed the clinical data of cancer patients undergoing oxaliplatin (OHP) treatment, and then studied the underlying mechanisms using a mouse model for CIPN. Analyses of 150 outpatients treated with OHP in Seichokai Fuchu Hospital identified a significant correlation between the severity of CIPN and plasma ALT, a marker of hepatic disorders. In mice, i.p. OHP at 5 mg/kg caused mechanical allodynia, which was prevented by an anti-HMGB1 antibody (AB) or soluble thrombomodulin (TM) capable of inactivating HMGB1. CCl_4 (1%, 5 ml/kg, i.p.) or ethanol (25%, 20 ml/kg x 3 for 2 days, p.o.) significantly increased ALT levels and tended to elevate HMGB1 levels in plasma. CCl_4 (every 2 days, 3 times) or ethanol (twice a day, 12 times) did not alter nociceptive threshold in naïve mice, but caused remarkable allodynia in the mice treated with OHP at 1 mg/kg, a subeffective dose, which was blocked by AB or TM. Thus, hepatic disorder is considered a risk factor for aggravation of OHP-induced CIPN in humans and mice, where HMGB1 might play a key role.

1-YIA-15

Schwann cell-dependent immune response is related to taxane-induced peripheral neuropathy

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Taxanes frequently cause chemotherapy-induced peripheral neuropathy (CIPN). However, the mechanisms underlying CIPN pathogenesis are not fully understood. We previously showed that taxanes preferentially impair Schwann cells (SCs) by inducing dedifferentiation. In this study, we further examined the roles of dedifferentiated SCs in the development of CIPN. We found that mRNA expression of an inflammatory factor, X, was increased in dedifferentiated SC culture or the mouse sciatic nerve after paclitaxel (0.01 μM) treatment or repeated i.p. injection of paclitaxel (20 mg/kg), respectively. Furthermore, murine macrophage cell line (RAW264.7) showed a chemotaxis response toward the conditioned medium of paclitaxel-treated SCs. Consistent with this, we found that the perineural application of an inflammatory factor derived from dedifferentiated SCs induced infiltration of macrophages into the sciatic nerve and mechanical hypersensitivity in mice. Taken together, our findings allow us to conclude that, in response to paclitaxel treatment, an inflammatory factor is released from dedifferentiated SCs to chemoattract macrophages. These SC-dependent macrophage migration may participate in paclitaxel-induced CIPN pathogenesis.

1-YIA-16

Advanced Glycation End product(AGE)-cholesterol-aggregated albumin induces dysfunction of mitochondria in mesangial cells

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We have previously demonstrated that advanced glycation end product (AGE)-aggregated albumin in the blood of diabetic mice induces acidification and apoptosis in mesangial cells(MCs).Our aim here is to clarify whether it could cause mesangial mitochondrial dysfunction. MCs were incubated with AGE-cholesterol aggregated albumin(ACAA),treated with TMRM(an indicator of mitochondrial membrane potential) or Caspase 3/7 reagent for apoptosis, and then analyzed by FACS.MCs were divided into 3 groups based on size and complexity; P1 small and complex, P2 large and complex, P3 small and simple.MC counts were similar in P1,P2 and P3 of the ACAA and EMEM(control) groups 3 hr after ACAA.Mitochondrial membrane potential(MMP) was 20 % higher in the ACAA group than in the EMEM group.On the other hand,MC counts in P1 were 80 % higher,and in P2 40 % lower,in the ACAA group than in the EMEM group 18 hr after ACAA.MMP in P1 was 45 % higher, and in P2 45 % lower,in the ACAA group than in EMEM group.The number of Caspase 3/7 positive MCs in ACAA group increased in P1,and total Caspase 3/7 fluorescein intensity in P1 was 3 times greater than that in P2 18 hr after ACAA.In the EMEM group,there was no difference in total Caspase 3/7 fluorescein intensity between P1 and P2.Mitochondria could be activated in MCs just after uptake of ACAA,and then gradually inactivated.Decreased MMP could lead to decreased levels of ATP and then MCs apoptosis and necrosis.

1-YIA-17

Inhibition of OASIS in podocytes suppressed diabetes-induced kidney dysfunction

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[Background] Diabetes is a major risk factor for chronic kidney disease (CKD). However, the mechanisms of diabetes-induced kidney injury remain to be fully elucidated. Here, we focused on old astrocyte specifically induced substance (OASIS), a transcriptional factor, because OASIS mRNA is increased in kidneys of CKD patients in the Nephroseq database. The aim is to determine the pathological roles of OASIS in diabetes-induced kidney injury.

[Methods/Results] C57BL/6J mice were injected with STZ to induce diabetes. Laser microdissection and immunoblotting revealed that OASIS was upregulated in glomeruli of STZ-treated mice. OASIS was detected in podocytes by immunohistochemical staining. To examine the roles of OASIS in podocytes, we generated podocyte-specific OASIS knockout mice (CKO). Mice were subjected to unilateral nephrectomy (UNx) before STZ injection to accelerate kidney injury. After UNx-STZ treatment, the level of serum creatinine (sCr) and the rate of kidney weight to body weight (Kw/Bw) got lower in CKO, compared with control (sCr (mg/dL): control; 0.85±0.11, CKO; 0.59±0.14, Kw/Bw (mg/g): control; 15.0±1.5, CKO;12.4±1.2, $P<0.05$, N=3-5).

[Conclusion] Podocyte OASIS could be a therapeutic target for the treatment of diabetic kidney disease.

1-YIA-18

Central angiotensin II type 1 receptors as a possible target for treatment of detrusor overactivity

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Purpose: We investigated the possible mechanism which central angiotensin II (Ang II) facilitates micturition reflex focusing on the Ang II type 1 receptor (AT1R), GABAR or corticotropin-releasing factor (CRF)R. And, we examined whether a centrally acting AT1R antagonist telmisartan (TEL) ameliorates the central Ang II induced stimulation of micturition reflex. **Materials and Methods:** Male Wistar rats were anesthetized with urethane, and cystomety was performed. TEL, $GABA_AR$ agonist (muscimol: Mus), $GABA_BR$ agonist (baclofen: Bac) or CRF1R antagonist (CP154526: CP) was icv administered before icv Ang II administration in the rats. Some rats were perorally administered with TEL (10 mg/kg/day) or no centrally acting AT1R antagonist (valsartan: Val, 10 mg/kg) for 8 days. Then, Ang II was icv administered in the rats. **Results:** TEL, Mus, Bac or CP significantly suppressed Ang II induced shortening of intercontraction interval (ICI). Chronic pre-treatment with TEL but not Val inhibited the Ang II induced shortening of ICI. **Conclusion:** Central Ang II can facilitate the micturition reflex via modulating the AT1R, GABAR or CRF1R. Blocking of the central AT1R might be a therapeutic target for treatment of detrusor overactivity.

1-YIA-19

Differences in hydrogen sulfide-induced relaxation of the bladder between hypertensive and normotensive rats

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Our recent report showed that hydrogen sulfide (H_2S) is a possible relaxation factor in the rat bladder. Because we have shown bladder dysfunctions develop in spontaneously hypertensive rats (SHRs), we compared effects of NaHS and GYY4137 (H_2S donors) on the bladder contractility and the micturition reflex, and H_2S contents in the bladder between 18-week-old male SHRs and normotensive Wistar rats (Wistars). Effects of NaHS (1×10^{-8} to 3×10^{-4} M) were evaluated on carbachol (10^{-5} M)-induced pre-contracted bladder strips. Under urethane-anesthesia, effects of intravesically instilled GYY4137 (10^{-8} to 10^{-6} M) on the rat micturition reflex were examined. Tissue H_2S contents were measured by the methylene blue method. NaHS-induced maximal relaxation was significantly higher in the strips of Wistars than those of SHRs. GYY4137 significantly prolonged intercontraction intervals in Wistars, but not in SHRs. The H_2S content in the bladder of SHRs was significantly higher than that in Wistars. These results suggest that H_2S-induced bladder relaxation in SHRs is impaired, which might be a cause of hypertension-mediated development of bladder dysfunctions, thereby resulting in a compensatory increase of the H_2S level in the SHR bladder.

1-YIA-20

Mitofusin2 promotes mitochondrial Ca^{2+} uptake in vascular smooth muscle cells.

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Mitochondrial Ca^{2+} uptake plays an important role for regulating Ca^{2+} signals in vascular smooth muscle cells (SMCs). However, Ca^{2+} affinity of mitochondrial Ca^{2+} uniporter (MCU) is very low. Mitofusin (Mfn) proteins are known to tether endoplasmic reticulum (ER) and mitochondria. Mfn1 is expressed in mitochondria membrane, whereas Mfn2 is localized in mitochondria and ER membranes. In the present study, we examined the physiological roles of Mfn proteins on Ca^{2+} signals in vascular SMCs. SMCs were enzymatically isolated from rat thoracic aorta. The cell proliferation was reduced by siMfn2, but not by siMfn1. The co-localization of mitochondria with sarcoplasmic reticulum (SR) was decreased by siMfn2, but not by siMfn1. In siMfn2-treated cells stimulated by vasopressin (AVP), the peak amplitude of $[Ca^{2+}]_{mito}$ was attenuated, whereas that of $[Ca^{2+}]_{SR}$ was not changed. The half duration of $[Ca^{2+}]_{cyt}$ increase was increased by siMfn2. These results indicate that Mfn2 regulates mitochondrial Ca^{2+} uptake without changing the parameters of Ca^{2+} release from SR. In conclusion, Mfn2 enhances Ca^{2+} signal through tethering SR to mitochondria, which promotes vascular SMC proliferation.

1-YIA-21

A Selective Bombesin Receptor Subtype 3 Agonist Promotes Weight Loss in Diet-Induced–Obese Rats With Circadian Rhythm Change

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Bombesin receptor subtype 3 (BRS-3) is an orphan G protein–coupled receptor. Based on the obese phenotype of male BRS-3–deficient mice, BRS-3 has been considered an attractive target for obesity treatment. Here, we developed a novel selective BRS-3 agonist (compound-A) and evaluated its antiobesity effects. Compound-A showed anorectic effects and enhanced energy expenditure in dietinduced–obese (DIO)-F344 rats. Moreover, repeated oral administration of compound-A for 7 days resulted in a significant body weight reduction in DIO-F344 rats. To investigate the underlying mechanisms of BRS-3 agonist effects, we focused on the suprachiasmatic nucleus (SCN), the main control center of circadian rhythms in the hypothalamus, also regulating sympathetic nervous system. Compound-A significantly increased the messenger RNA expression of Brs-3, c-fos, and circadian rhythm genes in SCN of DIO-F344 rats. On this basis, energy expenditure enhancement by compound-A may be due to a circadian rhythm change in central and peripheral tissues, enhancement of peripheral lipid metabolism, and stimulation of the sympathetic nervous system. According to these results, BRS-3 agonist might be a good option for obesity treatment with circadian rhythm change and increase of energy expenditure.

1-YIA-22

Nardilysin in hepatocyte senses nutrition and regulates adaptive thermogenesis in brown adipose tissue

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Thermogenesis is enhanced not only by cold exposure but also by feeding, which is considered as a partial defense mechanism against obesity. However, the molecular mechanism of diet-induced thermogenesis has remained unclear. Here we found that metallopeptidase nardilysin (NRDC) expression in liver is increased by fasting and decreased by re-feeding in wild-type mice. To elucidate the liver-specific role of NRDC in energy metabolism, we established hepatocyte-specific NRDC deficient mice (LKO). These mice showed intriguing phenotypes including 1) elevation of thermogenic genes in BAT, 2) decrease in lipid accumulation in BAT, and 3) increase in whole-body energy expenditure. These results suggested that the loss of NRDC in hepatocyte enhances adaptive thermogenesis in BAT by an inter-organ metabolic network. Notably, the phenotypic difference between control and LKO was completely eliminated by hepatic vagotomy or elevation of ambient temperature to thermoneutral range (30℃). Furthermore, LKO showed a significant increase in skin blood flow of the plantar at room temperature (23℃), suggesting that heat dissipation is enhanced in LKO. Taken together, these results indicate that hepatic NRDC regulates skin blood flow, thus heat dissipation via nervous system. BAT thermogenesis was then enhanced to compensate for the heat loss. In conclusion, NRDC, a novel sensor of nutrition, mediates diet-induced thermogenesis.

1-YIA-23

The importance of heparan sulfate in 3T3-L1 cells and white adipose tissues

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Heparan sulfate (HS) is a highly sulfated glycosaminoglycan distributed on the cell surface and in the extracellular matrix. HS is involved in diverse biological events including embryonic development, angiogenesis and tumor metastasis. Recent studies revealed the pathophysiological involvement of HS in diabetes and we showed HS had a great impact on pancreatic β-cell function. However, the roles of HS in adipose tissue, an important tissue for glucose metabolism, remained to be elucidated.
First, we evaluated the roles HS in 3T3-L1, a cell line of mouse adipocytes. Biochemical assays indicated that HS promoted differentiation of 3T3-L1 possibly via enhancement of FGF signaling. Next, we generated and phenotyped white adipocyte-specific HS-deleted mice (cKO). Several differentiation markers in cKO adipocytes were decreased, emphasizing the important role of HS in adipocyte differentiation. We also confirmed the reduction in gene expression of essential factors for insulin-dependent glucose transport in cKO adipocytes. Indeed, glucose tolerance test revealed glucose intolerance due to insulin resistance in cKO mice. These results demonstrated that HS played an important role in adipocyte differentiation, leading to normal insulin sensitivity and glucose homeostasis.

1-YIA-24

The effect of pressure culture on the differentiation of 3T3-L1 preadipocyte

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Background:It is known that body fat mass decreases by exercise under hyperoxia. Besides, hypoxia plays an important role in maintenance of stem cell niche in regenerative medicine field. Although it is obvious that the oxygen partial pressure affects cell proliferation, the relationship between pressure culture condition and cell signal has hardly been elucidated. Control of adipocyte proliferation is an indispensable technology in regenerative medicine to treat obesity and adipose cell transplantation as a fundamental therapy for lipodystrophy. So, in this study, we examined the effect of adipocyte differentiation under the high-pressure cultivation. Methods:3T3-L1 cells were kept under the normal pressure condition or pressurized condition during differentiation for 14 days. Intracellular lipid droplet was evaluated by Oil Red O staining and expressions of adipogenic genes were determined by real time PCR. Results & Conclusions:Lipid droplet and mRNA level of adipogenic genes decreased under the high-pressure cultivation compared to the normal pressure. These data suggest that the high-pressure condition prevented adipocyte differentiation by suppressing expression of adipogenic genes, which induced a decrease in intracellular lipid droplet.

1-YIA-25

Roles of nuclear receptor 4a family in maintenance of stemness of murine adipose-derived stem cells

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Obesity is associated with proliferation and differentiation of adipose-derived stem cells (ADSCs) into mature adipocytes. Nutritional stimuli induce ADSCs proliferation and differentiation, and this process is well established because master regulators of adipogenic differentiation, C/EBPalpha and PPARgamma were identified. However, under normal condition, molecular mechanisms to maintain stemness of ADSCs are largely unknown.

To identify genes essential for the maintenance, microarray analysis was performed on murine ADSCs and 4-day cultured ADSCs (preadipocytes). Among the 223 up-regulated transcriptional factor genes in ADSCs, we focused on nuclear receptor 4a (Nr4a) family, which play diverse roles including metabolic processes. Nr4a-overexpressed preadipocytes showed reduced accumulation of lipid droplet and decreased expressions of C/EBPalpha and PPARgamma. ChIP analysis confirmed that Nr4a directly bound to C/EBPalpha and PPARgamma promotor. Nr4a family is induced by multiple signals including cyclic AMP in a cell type-specific manner. Application of a cAMP analogue to ADSCs induced Nr4a expressions and decreased expressions of PPARgamma. These data suggested that Nr4a inhibited ADSCs differentiation in a cAMP-dependent manner.

1-YIA-26

Development of FABP3 ligands inhibiting α-synuclein oligomerization induced by arachidonic acid

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【Introduction】In Parkinson`s disease (PD), α-synuclein (αSyn) accumulation and inclusion triggers dopamine neuronal death and synapse dysfunction in vivo. We previously reported that fatty acid-binding protein 3 (FABP3) is highly expresses in dopaminergic neurons and aggravates αSyn oligomerization when exposure to 1-Methyl-1,2,3,6-tetrahydropiridine (MPTP) in vivo and in vitro. We here discovered FABP3 ligands inhibiting α-synuclein oligomerization induced by arachidonic acid (AA).

【Method 】FABP ligands were modified from FABP4 inhibitor BMS309403 and assessed their inhibitory action on AA-induced α-synuclein oligomerization using FABP3 and αSyn co-overexpressed Neuron2A cells. αSyn oligomerization levels were measured using western blotting assay and immunohistochemical analyses.

【Summary】AA treatment triggered αSyn oligomerization in Neuro2A cells in FABP3-dependent manner. A potent FABP3 ligand 1 totally blocked αSyn oligomerization and aggregation induced by FAPB3 and AA. In addition, ligands 7 and 8 also elicited inhibition of αSyn oligomerization in Neuro2A cells. Taken together, the new FABP3 ligand is attractive therapeutic candidate for Parkinson and Lewy body diseases.

1-YIA-27

The involvement of progranulin for α-synuclein reduction through autolysosome formation

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Progranulin is a multipotent protein that contributes to various pathology such as inflammation and tumorigenesis. Absence of progranulin gene causes the onset of frontotemporal lobar degeneration (FTLD) and neural ceroid lipofuscinosis. Recently, it has been reported that some Parkinson's disease patients with α-synuclein lesions have a progranulin gene mutation. However, the relationship between progranulin and α-synuclein accumulation mechanism is unclear. We examined the effect of progranulin against α-synuclein accumulation.

We evaluated progranulin effects against MPP^+ damage in human derived neuroblastoma cells (SHSY-5Y cells). A-synuclein and autophagy related factors (AMPK, mTOR, LC-3, p62) were evaluated by Western blot. To clarify autolysosome formation, we used DAL-Green, specific autolysosome detector.

A-synuclein expression was reduced by progranulin treatment. On the other hand, AMPKor mTOR activation were not changed. Furthermore, LC-3II/LC3-I ratio and p62 expression were reduced. These results indicate that progranulin ameliorated autolysosome formation decreased by MPP^+.

These findings indicate that progranulin promotes autophagy degradation by inhibiting autolysosome formation and reduces α-synuclein accumulation.

1-YIA-28

The preventive approach for Alzheimer's disease based on the novel neprilysin regulator

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One of the pathological hallmarks of Alzheimer's disease (AD) is senile plaque composed of amyloid-β peptide (Aβ) in the brain. Neprilysin (NEP) is a potent Aβ degrading enzyme. Therefore, identification of the mechanism for NEP activity may lead to development of a preventive approach for AD. Previously we showed that somatostatin (SST) regulates NEP activity. However the molecular mechanism by which SST regulates NEP in the brain is unclear. Recently, We found that cortex/hippocampus-basal ganglia communication is important to upregulate NEP upon SST stimulation. The aim of this study is elucidation of the molecular mechanism of NEP activity based on the cell-cell communication. Firstly proteomics using primary neurons treated with SST identified α-endosulfine (ENSA), an endogenous ligand for a potassium channel, as a novel NEP regulator. To investigate the function of ENSA *in vivo*, we generated ENSA knock out mice using CRISPR/Cas9 and found that deficiency of ENSA increased NEP activity in the brain. Finally administration of Diazoxide which is a potassium channel modulator decreased amyloid pathology in AD model mice mediated by activation of NEP. Thus, we found a novel preventive approach for AD based on the cell-cell communication.

1-YIA-29
Identification of amyloid breakers for amyloidosis

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Amyloidosis causes organ dysfunction due to deposition of β-sheet structured amyloid fibrils in multiple organs. Drugs that break up amyloid fibrils (amyloid breakers) is considered as promising therapy for amyloidosis, but none is yet clinically available. Recently, we performed *in vitro* fluorescence-based high throughput screening (HTS) (1280 compounds) to identify small molecules that interact with a mutant transthyretin (TTR), a causative protein of hereditary ATTR (ATTRm) amyloidosis. Here, we focused on the HTS-derived nineteen hit compounds and identified two compounds (B and R) that decrease preformed mutant TTR amyloid fibrils. We next validated 113 analogs harboring common basic chemical structure with compound B, and demonstrated that 12 compounds significantly disrupt preformed mutant TTR amyloid fibrils. Importantly, among these 12 compounds, several compounds also broke preformed amyloid fibrils derived from amyloid-β (Aβ) and tau proteins, well-known amyloid causative proteins in Alzheimer's disease. Finally, some of the hit compounds are the component of natural products with low cytotoxicity. Overall, the study will be of great interest for the development of not only therapeutic drugs but also healthy supplements that are applicable for amyloidosis.

1-YIA-30
Influence of meningeal lymphatic vessels on brain mechanisms

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The central nervous system has been considered as an organ devoid of lymphatic vessels. Meningeal lymphatic vessels were discovered and it is thought that elucidating mechanism of these vessels contributes to the studies on neurodegenerative diseases such as Alzheimer's disease and multiple sclerosis. Previous researches focused on material flow in the brain and excretion of proteins and immune cells. There are few researches directly investigating the relationship between the brain and meningeal lymphatic vessels. Our research focused on structure of brain cells and neural activities. Meningeal lymphatic vessels connect to the deep cervical lymph nodes (dcLNs). Ligating efferent lymphatic vessels of dcLNs lead to the reduction of cerebrospinal fluid (CSF) drainage. Electrocorticography (ECoG) was recorded for 8 weeks and glial cells and synapses were immunostained in mice 1, 4 and 8 weeks after ligation. Results showed that reducing lymphatic flow concerned neural activities.

1-YIA-31

SIRT1 in the skeletal muscle maintains muscle function and attenuates muscle membrane injury

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[Background and Aim] SIRT1, an NAD^+-dependent protein deacetylase, exerts cytoprotective effects. We previously reported that resveratrol, an activator of SIRT1, attenuates skeletal muscle pathology in a mouse model of Duchenne muscular dystrophy. The purpose of this study was to elucidate the function of SIRT1 in skeletal muscle using muscle-specific SIRT1 knockout mice (SIRT1MKO).

[Method and Result] Treadmill running distance and inverted net hanging time were significantly shorter in SIRT1MKO than those in wild-type mouse (WT). Blood level of creatine kinase, a marker of muscle membrane injury, after treadmill exercise was significantly higher in SIRT1MKO (2201 ± 407 U/L) than WT (481 ± 99 U/L). Furthermore, Evans blue uptake into muscle cells, which reflects plasma membrane rupture, after treadmill was increased in SIRTMKO than that in WT (1.8% vs. 0.5%, $P<0.05$). Histological analyses of quadriceps muscle showed that central nuclei, an indicator of muscle regeneration, and atrophied muscle fibers with ($<1,500\ \mu m^2$) were significantly increased in SIRT1MKO compared to those in WT.

[Conclusion] These results suggest that SIRT1 maintains muscle function and attenuates muscle membrane injury.

1-YIA-32

Drug development of fibrodysplasia ossificans progressiva (FOP) focused on constitutive activation of FOP-ACVR1

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Fibrodysplasia ossificans progressiva (FOP) is a rare genetic disease characterized by extraskeletal bone formation through endochondral ossification. FOP patients harbor gain-of-function mutations in ACVR1 (also known as ALK2), a type I receptor for bone morphogenetic protein (BMP). Despite numerous studies, no drugs have been approved for FOP. Here, we developed a high-throughput screening (HTS) system focused on the constitutive activation of FOP-ACVR1 by utilizing a chondrogenic ATDC5 cell line that stably expresses FOP-ACVR1. After HTS 5,000 small molecule compounds, we identified two hit compounds that are effective at suppressing the enhanced chondrogenesis of FOP patient-derived iPSCs (FOP-iPSCs) and suppressed the HO of multiple model mice, including FOP-ACVR1 transgenic mice and HO model mice utilizing FOP-iPSCs. Furthermore, we revealed that one of the hit compounds is a mTOR signaling modulator that indirectly inhibits mTOR signaling. Our results demonstrate that these hit compounds could contribute to future drug repositioning and the mechanistic analysis of mTOR signaling.

1-YIA-33
Visualizing the rotation of single-actin filaments in living cells

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Theorientation of actin filaments regulates protrusion and contractile movement of cells because it determines the direction of actin elongation and myosin movement. Little is known about the orientation change of a single actin filament in living cells because of the lack of the method to probe the orientation of actin filaments in living cells. Here we report a method to visualize the orientation of single actin filament. We labeled N and C-termini of tropomyosin with different fluorescent dyes, and introduced it into living cells by electroporation. We performed dual fluorophore-single molecule localization analysis and orientation of actin filaments was determined by the relative position of N and C-termini. We observed the orientation of actin filaments, which flow near focal adhesions(FAs), and found that not only actin filaments rotate rapidly near the FAs, but also they rotate inwardlyin the frontal region of FAs. Actin filaments are also disrupted in the anterior region of FAs. The actin remodeling zone surrounding FAs may be the place for reconstructing the FAs-linked actin network.

1-YIA-34

High-resolution plasma membrane-selective imaging by second harmonic generation

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The plasma membrane is the site of intercellular communication and subsequent intracellular signal transduction. The specific visualization of the plasma membrane in living cells, however, is difficult using fluorescence-based techniques owing to the high background signals from intracellular organelles. In this study, we show that second harmonic generation (SHG) is a high-resolution plasma membrane-selective imaging technique that enables multifaceted investigations of the plasma membrane. In contrast to fluorescence imaging, SHG specifically visualizes the plasma membrane at locations that are not attached to artificial substrates and allows high-resolution imaging because of its subresolution nature. These properties were exploited to measure the distances from the plasma membrane to subcortical actin and tubulin fibers, revealing the precise cytoskeletal organization beneath the plasma membrane. Thus, SHG imaging enables the specific visualization of phenomena in the plasma membrane with unprecedented precision and versatility and should facilitate cell biology research focused on the plasma membrane.

1-YIA-35

PERO: A stress-free, quantitative oral administration method through voluntary licking behavior

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Oral administration is widely used in pharmacological experiments. However, the most common method, oral gavage, causes stress responses through restraint and insertion of a needle into the stomach, often affecting subsequent animal behaviors such as sleep/wake. Here, we developed a new method of voluntary oral administration: PERO. In PERO, we used a mixture of viscous paste foods (e.g. sweetened condensed milk) and a solvent (dimethyl sulfoxide) as vehicle. Mice rapidly and completely consumed the vehicle by licking, and the behavior persisted for 4 weeks when repeated daily. Plasma corticosterone levels showed that our method was significantly less stressful than oral gavage. Moreover, we demonstrated the utility of the method through an assay for sleep-inducing effects of a water-insoluble orexin antagonist. In conclusion, PERO will be useful especially in behavioral neuroscience vulnerable to stress responses, and will improve animal welfare.

1-YIA-36
Screening of antifibrotic compounds using myofibroblasts

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Idiopathic pulmonary fibrosis (IPF) is a chronic and progressive disease of unknown cause. Under the pathogenic environment, myofibroblasts (MyoFs) mainly differentiated from fibroblasts play a key role in lung fibrogenesis. Established MyoF has been considered as an irreversible phenotype, but recently shown to dedifferentiate to fibroblast. This feature is desirable in development of pharmacologic strategy against IPF because most patients with IPF appear to accumulate pathogenic MyoFs in their lungs at the time of clinical presentation. Therefore, we have established several strains of primary cultured MyoFs from the fibrotic lungs of patients. Using our MyoF assay system, the drug library for compounds that inhibit epigenetics-related signals was screened by monitoring downregulation in expression of collagen and MyoF markers, α-SMA and ED-A fibronectin. Through this assay, we found in vitro that a certain histone methyltransferase inhibitor potently dedifferentiated MyoFs. In addition, intratracheal administration with the compound at the early fibrotic stage of bleomycin-injured lung successfully ameliorated lung fibrosis in mice. We will discuss the mechanism by which the compound affects pathogenic myofibroblast under lung fibrogenesis.

1-YIA-37

Interaction between IL-17A and IL-13 is involved in steroid-resistant increase in airway mucus production.

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Mucus hyperproduction is a hallmark of chronic airway inflammatory diseases, such as asthma and COPD. Mucus production in a population of patients with severe asthma does not respond to steroids. However, the underlying mechanism of this steroid-resistance remains incompletely understood. In our pharmacological study with house dust mite (HDM)-sensitized asthmatic mice, we have found that MUC5AC mucus gene expression was steroid resistant, although the number of eosinophil was significantly decreased. In these mice treated with DEX, the expression level of IL-17A and IL-13 was considerably high, as well as MUC5AC. We also found that intratracheal administration of both IL-17A and IL-13 was sufficient to induce steroid-resistant MUC5AC production in lung, whereas IL -17A or IL-13 alone was not. Furthermore, HDM-induced steroid-resistant MUC5AC production was partially reversible with DEX in mice lacking IL-17A. Collectively, these results suggest that the coordinated action of IL-17A and IL-13 mediates steroid-resistant mucus production in mouse model of asthma.

1-YIA-38

Delayed colonic motilty in functionally deficient mice of myosin phosphatase inhibitory protein, CPI-17

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Background RhoA/Rho kinase and PKC/CPI17mediated signaling can inhibit myosin phosphatase to induce contraction in smooth muscles. However it is still unclear how the signaling plays an important role to regulate gastrointestinal motility *in vivo*.
Aim Intestinal and colonic contractile and transit abilities were tested using CPI17 deficient (KO), phospho-inactive mutant CPI17knock in (TA) and wild type mice (WT).
Methods Isometric force and phosphorylation of Myosin light chain (MLC) and CPI17 stimulatedwith Carbochol(Cch)were measured. Transit abilities were observed by FITC dextran and dextran beads method, respectively.
Results High concentration of KCl induced contractions were no difference among WT, KO and TA of ileal and colonic circular muscles. However the sustained contraction by Cch of ileum and colon in KO and TA were decreased compared with WT. The MLC phosphorylation level was also lower in KO and TA than WT. Ileal transit ability did not change in KO and TA compared with WT.In contrast,colonic transit in KO and TA were significantly delayed compared with WT.
Conclusion CPI17 is important to maintain the sustained contraction of gastrointestinal tract due to receptor stimulation *in vitro*. In addition, the PKC/CPI17 pathway is more important for maintenance in colon transit ability than ileal part *in vivo.*

1-YIA-39

Carbon tetrachloride mediated liver fibrosis is alleviated in α7 nicotinic acetylcholine receptor knockout mice

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Background: Cirrhosis is a condition come from excessive liver fibrosis and followed by serious second diseases. 1.3 million people are died of cirrhosis in a year but there is no effective therapeutic medicine. α7 nicotinic acetylcholine receptor (α7nAChR), initially found a receptor related to neurotransmission, expresses on immune cells and activation of this receptor leads anti-inflammatory effect. However, there is few reports showing the relationship between α7nAChR and fibrosis.
Aim: Using α7nAChR knocked out mice (α7 KO), we investigated whether α7nAChR has any effect on liver fibrosis.
Methods: Liver fibrosis model mice were established with carbon tetrachloride (CCl_4, 1 ml/kg, twice a week). The amount of collagen and pro-fibrotic mRNA expressions in livers were measured at 1.5 and 4 weeks.
Results: α7 KO treated with CCl_4 showed significant decrease in liver fibrosis at 4 weeks compared to wild type mice (WT). Furthermore, mRNA expressions of Acta2, TGF-β1 and Col1a1 in α7 KO were significantly lower than WT at 1.5 weeks.
Conclusion: Increase of pro-fibrotic mRNA expression and liver fibrosis induced by CCl_4 were alleviated in α7nAChR KO mice. These data suggested that α7nAChR might be a therapeutic target for cirrhosis.

1-YIA-40

MITOCHONDRIA-TARGETED MITO-TEMPO AS A PROMISING ADJUVANT AGAINST DRUG-INDUCED LIVER INJURY MODELS

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N-acetyl-*p*-aminophenol (APAP), Concanavalin A (ConA) and Carbon tetrachloride (CCl_4) exposure generated drug induced liver injury models. Overdose of APAP is the most common cause of acute liver failure, as it induced mitochondrial oxidative stress and hepatic necroptosis. Also, ConA and CCl_4 are causing immune-mediated liver injury and centrilobular necrosis, respectively. We conducted this study to evaluate the effects of Mito-TEMPO (Mito-T) on acute liver injury models in mice. An injection of Mito-T (20 mg/kg) after 1h of APAP (400 mg/kg) administration markedly inhibited the elevation of serum transaminase activity but was not able to attenuate the ConA (12.5 mg/kg) and CCl_4 (0.025 mL/kg) hepatotoxicity in C57BL/6J mice. Mito-T significantly reduced the parameters of APAP derived oxidative stress, such as mitochondrial radical production and nitrotyrosine formation in mouse liver; though the decreased glutathione (GSH) level and c-Jun N-terminal Kinases (JNK) activation were not suppressed in liver. In addition, Mito-T was hepatoprotective after 4 hours of APAP ingestion, whereas, N-acetyl-L-cysteine, the only recognized therapeutic agent against APAP hepatotoxicity, became less effective with the time lapse.
We demonstrated that Mito-T alleviates mitochondrial oxidative stress and suppresses the APAP-induced liver injury in mice. The results suggest that Mito-T could be a promising novel therapeutic agent for APAP overdosed liver injury.

2-YIA-01

Optogenetic regulation of astrocytes affects learning and memory

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Astrocytes are crucial for synaptic plasticity and memory formation. Astrocyte-derived factors, such as D-serine and lactate, have been suggested to enhance learning and memory likely via modulating NMDA receptor functions in neurons. However, the intracellular mechanisms of astrocytes that release these factors are not fully clarified. Here we adopted an optogenetic approach to regulate the intracellular cyclic AMP (cAMP), a major second messenger, specifically in astrocytes *in vivo.* We developed a transgenic mouse line in which astrocytes express photoactivated adenylyl cyclase (PAC), a protein that rapidly changes its conformation and synthesizes cAMP from ATP in response to blue light. In these mice, we optogenecically modulated the intracellular levels of cAMP in hippocampal astrocytes and investigated the effect of a prolonged cAMP elevation on spatial memory. We found that a long-term cAMP elevation in astrocytes after training session facilitated the memory fading, whereas a short-term cAMP elevation enhanced memory formation. We also found that astrocytic cAMP facilitates synaptic plasticity through activating NMDA receptors, which may underlie both memory fading and enhanced memory formation. Thus, our results suggest that astrocytic cAMP signaling modulates hippocampus-dependent learning and memory.

2-YIA-02
The CaMKII-Tiam1 complex in memory storage process

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A stimulation inducing long-term potentiation (LTP) of synaptic transmission induces a persistent expansion of dendritic spines, phenomena recognized as structural LTP (sLTP). Actin cytoskeleton plays an essential role in this process. We previously proposed the persistent interaction between CaMKII and Tiam1, an activator of Rac is a critical mediator of actin regulation. Interestingly, the formation of the complex locks CaMKII into an active conformation, which in turn maintains the activity of Tiam1 though phosphorylation. This makes Rac1 activity persist in a stimulated spine. Therefore, the CaMKII-Tiam1 complex plays a pivotal role in sLTP.

To understand the significance of the CaMKII-Tiam1 complex in vivo, we generated Tiam1 knocked-in mice where critical residues for CaMKII binding were mutated into alanines. In the KI brain, Rac1 activity was specifically reduced compared with WT littermate. Also sLTP was abolished in hippocampal neurons from KI. Gross appearance of brain structure and spine density was normal in KI mice. The KI mice showed decreased object recognition memory 7 days after training while the other behavioral tests were normal. Thus, the CaMKII-Tiam1 interaction is not only crucial for sLTP but also requires for memory storage.

2-YIA-03

Oral administration of food-derived hydrophilic antioxidant ergothioneine enhances object recognition memory and promotes neuronal maturation in murine hippocampus

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The aim of the present study was to examine enhancement of learning and memory by oral administration of ergothioneine (ERGO), which is a hydrophilic antioxidant highly contained in golden oyster mushrooms and other foods, and systemically absorbed by its specific transporter OCTN1/SLC22A4 in daily life, with an aim to clarify its possible role as a neurotropic compound. After oral administration of ERGO in normal mice, the novel object test revealed a longer exploration time for the novel object than for the familiar object. Similar result was also confirmed in mice ingested with ERGO-free diet. Dietary-derived ERGO is present in the body without the administration, but the ERGO administration led to modest (3～4 times) increase in its concentration in plasma and hippocampus. Exposure of cultured hippocampal neurons to ERGO elevated the expression of the synapse formation marker, synapsin I, and neurotrophin-3 and -5. The elevation of synapsin I was inhibited by tropomyosin receptor kinase inhibitor K252a. Thus, oral intake of ERGO may enhance object recognition memory, and this could occur at least partially through promotion of neuronal maturation in the hippocampus.

2-YIA-04

An adenosine A_{2A} receptor antagonist improves multiple symptoms reflecting obsessive-compulsive disorder

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Obsessive-compulsive disorder (OCD) is a psychiatric disorder characterized by repetitive inappropriate thoughts (obsessions) and behaviors to get rid of obsessions (compulsions). Although selective serotonin reuptake inhibitors (SSRIs) are the first-choice treatment for OCD, response rates to SSRI treatment vary between symptom dimensions. In this study, to find a therapeutic target for SSRI-resilient OCD symptoms, we evaluated treatment responses of quinpirole sensitization-induced OCD-related behaviors. Chronic SSRI administration rescued the cognitive inflexibility and the hyperactivity in the lateral orbitofrontal cortex (lOFC), while repetitive behavior was not improved by SSRI. D_2 receptor signaling in the central striatum (CS) was involved in SSRI-resistant repetitive behavior. An adenosine A_{2A} antagonist, istradefylline rescued abnormal excitatory synaptic function in the CS indirect pathway medium spiny neurons of sensitized mice and also alleviated both of the QNP-induced OCD-related behaviors with only short-term administration. These results provide a new insight into therapeutic strategies for SSRI-resistant OCD symptoms and indicate the potential of A_{2A} antagonists as a rapid-acting anti-OCD drug.

2-YIA-05

Activation of neural projection from the anterior cingulate cortex to the periaqueductal gray facilitates reward-seeking behavior.

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It is crucial for animals to select an appropriate behavior in a conflict situation where they face both positive and negative motivation. But the neural circuit mechanism underlying behavioral selection in a conflict situation is unclear. We set up a behavioral paradigm in which mice need to explore an experimental context where they might receive electric shocks in order to obtain sucrose solution. Using c-Fos expression as a marker of neuronal activation, we found that the anterior cingulate cortex (ACC) and periaqueductal gray (PAG) were activated in the conflict situation. Anterograde and retrograde tracing with AAV-CaMKII-eYFP and Fluoro-Gold, respectively, revealed dense projections from the ACC to the PAG. To record ACC-PAG activities from behaving mice, a genetically encoded calcium indicator, GCaMP6, was expressed selectively in the ACC-PAG pathway and its fluorescence was detected through an optic fiber. ACC-PAG activity was associated with sucrose-seeking behavior. Furthermore, optogenetic activation of the ACC-PAG pathway shortened the latency to obtain sucrose. These results suggest that ACC inputs to the PAG facilitate reward-seeking behavior in the conflict context.

2-YIA-06

Periostin mediates cardiac dysfunction through activating right ventricular fibroblasts

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The pathophysiological role of periostin (POSTN), expression of which is increased in right ventricles of monocrotaline (MCT)-induced pulmonary arterial hypertension (PAH) model rats, has not been clarified. We investigated the effect of POSTN on inducible nitric oxide synthase (iNOS) expression, which causes cardiac dysfunction, in right ventricular fibroblasts (RVFbs). PAH model rats were produced by an intraperitoneal injection with MCT (60 mg/kg). In RVFbs isolated from PAH model rats, the iNOS expression and phosphorylation of extracellular signal-regulated kinase (ERK) 1/2, c-Jun N-terminal kinase (JNK) and nuclear factor (NF)-kB were higher than RVFbs isolated from control rats. Recombinant POSTN increased iNOS expression and NO production in RVFbs isolated from normal rats, which were prevented by a pharmacological inhibition of ERK1/2, JNK or NF-kB. The culture medium of recombinant POSTN-stimulated RVFbs suppressed L-type Ca^{2+} channel (LTCC) activity in H9c2 cardiomyoblasts. We demonstrated that POSTN increased iNOS expression and subsequent NO production in RVFbs. The enhanced NO production in RVFbs might be associated with the right ventricular dysfunction via the suppression of LTCC activity of cardiomyocytes in PAH.

2-YIA-07

Canstatin, a cleaved fragment of type IV collagen α2 chain, prevents heart failure after myocardial infarction in rats

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Canstatin, a cleaved C-terminal fragment of type IV collagen α2 chain, inhibits hypoxia-induced apoptosis in H9c2 cardiomyoblasts. After myocardial infarction, the expression of canstatin is decreased in the infarcted area. We investigated whether the canstatin-treatment exerts a cardioprotective effect on heart failure after myocardial infarction in rats. The myocardial infarction model rats induced by ligating left anterior descending artery were intraperitoneally injected with recombinant canstatin (20 μg/kg/day) or vehicle for 28 days after the operation. Echocardiographic analysis showed that canstatin-treatment significantly inhibited cardiac dysfunction and tended to inhibit left ventricular dilation. Canstatin did not change the ratio of infarcted area to left ventricular area. In the histological analysis of non-infarcted area, canstatin inhibited hypertrophy of cardiomyocytes (using hematoxylin and eosin staining) and interstitial fibrosis (using picro-sirius red staining). The present study for the first time demonstrated that chronic recombinant canstatin-treatment prevents heart failure after myocardial infarction through the inhibition of cardiac hypertrophy and fibrosis in non-infarcted area.

2-YIA-08

2,5-Dimethylcelecoxib prevents isoprenaline-induced cardiac hypertrophy and fibrosis via the activation of glycogen synthase kinase-3

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Background- We previously reported that 2,5-dimethylcelecoxib (DMC) activated glycogen synthase kinase-3 (GSK-3), a negative regulator of cardiac hypertrophy, in mice. In this study, we examined the effects of DMC on isoprenaline (ISO)-induced cardiac hypertrophy and fibrosis using *in vivo* and *in vitro* systems.

Methods- ISO (20 mg/kg/day) was administered to C57BL/6J mice using an osmotic pump, and DMC (1,000 ppm) was added into feed for 14 days. Mice were divided into 3 groups: Control, ISO and ISO+DMC. Primary neonatal rat ventricular cardiomyocytes (NRVCs) and adult rat cardiac fibroblasts (RCFs) were pretreated with DMC (3-20 mmol/L) from 1 hour before incubation with ISO (5 mmol/L) for 24 hours. NRVC sizes and RCF proliferation were measured and proteins were examined.

Results- DMC prevented hypertrophy and fibrosis *in vivo*. DMC attenuated the enlargement of NRVCs by activating GSK-3 and suppressing β-catenin and mTOR. DMC also attenuated RCF proliferation by activating GSK-3 and suppressing cyclin D1. The direct involvement of GSK-3 was verified using a GSK-3 inhibitor SB216763.

Conclusion- DMC prevented cardiac hypertrophy and fibrosis by activating GSK-3 and modulating downstream proteins.

2-YIA-09

A bacteria-derived ACE2-like enzyme suppresses cardiac remodeling and dysfunction in mice.

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Angiotensin-converting enzyme 2 (ACE2) is a negative regulator of renin-angiotensin system and has the beneficial effects on the cardiovascular diseases. We elucidate that the B38-CAP, a carboxypeptidase derived from Paenibacillus sp.B38, has ACE2-like enzymatic activity. In silico analysis revealed the structural similarity between B38-CAP and rhACE2 despite the lack of obvious sequence homology. In vitro recombinant B38-CAP protein catalyzed the conversion of angiotensin II to angiotensin 1-7 with the same potency as rhACE2. Treatment with B38-CAP reduced plasma angiotensin II levels and suppressed angiotensin II-induced hypertension, cardiac hypertrophy and fibrosis in mice. Moreover, continuous infusion of B38-CAP inhibited pressure overload-induced pathological hypertrophy, myocardial fibrosis, and cardiac dysfunction in mice. Importantly, B38-CAP treatment did not induce overt toxicity of liver and kidney. B38-CAP is an ACE2-like carboxypeptidase, which is functional in vitro and in vivo. B38-CAP could be a novel therapeutics in cardiovascular diseases.These results suggest that the strategy to find molecules of convergent evolution might be effective for drug development, such as ‘generic' protein preparation of functional enzymes.

2-YIA-10

Runx2-expressing myeloid cells ameliorate post-infarct cardiac remodeling as a novel cardioprotective subset

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【Background】
Runx2 is a critical regulator of osteoblast differentiation; however, the pathophysiological significance of Runx2 in cardiac homeostasis remains to be elucidated.
【Methods and Results】
Murine myocardial infarction (MI) was generated by ligating the left coronary artery. Quantitative RT-PCR and immunoblot analyses revealed the up-regulation of Runx2 mRNA and protein in post-infarct myocardium. Immunofluorescent staining and flow cytometric analyses showed that Runx2 was expressed in heart-infiltrating $CD11b^{+}$ myeloid cells after MI. To analyze the biological functions of Runx2 during post-infarct cardiac remodeling, myeloid cell-specific Runx2 deficient mice (CKO mice) were exposed to MI. MI induced severe heart failure and lung congestion in CKO mice. CKO mice showed exacerbated cardiac fibrosis and function, respectively after MI. RNA sequence analyses demonstrated that myeloid expression of Runx2 regulated the gene expression responsible for vascular functions. Consistently, capillary density was decreased in CKO mice, proposing the importance of Runx2-expressing myeloid cells in cardiac repair. Single-cell RNA sequence analyses showed that Runx2-expressing cells did not coincide with either M1 and M2 macrophages.
【Conclusion】
Runx2-expressing myeloid cells prevented post-infarct cardiac remodeling as a novel cell population.

2-YIA-11

Contribution of Kir2.1 channels to cell functions in mouse bone marrow-derived macrophage.

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Intracellular Ca^{2+} signal controls various cell functions such as gene expression, cell death, proliferation, and migration. In immune cells, the activation of K^+ channels promotes Ca^{2+} entry and therefore regulates cell functions. Inwardly rectifying K^+ (Kir) channels play important roles in the formation of resting membrane potential in various types of cells. Kir2.1 channel is expressed in macrophages, however, the contribution of Kir2.1 channels to the pathophysiological functions is unclear. In this study, we examined the pathophysiological roles of Kir2.1 channels in murine bone marrow-derived macrophage (BMDM). The resting membrane potential in BMDM significantly depolarized by 100 μM Ba^{2+} (a Kir blocker). The resting $[Ca^{2+}]_i$ and store-operated Ca^{2+} entry (SOCE) induced by 1 μM thapsigargin were significantly reduced by 100 μM Ba^{2+}. Neither application of Ba^{2+} nor ML133 (a Kir2 selective blocker) affected differentiation of myeloid progenitor cell into BMDM, phagocytosis, and proliferation. On the other hand, migration of BMDM was significantly inhibited by these Kir2.1 inhibitors. These results suggest that the activity of Kir2.1 channels regulates resting membrane potential and $[Ca^{2+}]_i$, and thus promotes migration to inflammatory regions in macrophage.

2-YIA-12

HIF-1α-mediated up-regulation of $K_{2P}5.1$ K^+ channels in $CD4^+$ T cells of inflammatory bowel disease model mice

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The alkaline pH-activated $K_{2P}5.1$ K^+ channel contributes to the setting of the resting potential and the control of Ca^{2+} signaling. $K_{2P}5.1$ is up-regulated in $CD4^+$ T cells from patients of autoimmune diseases such as rheumatoid arthritis and multiple sclerosis. We have reported that $K_{2P}5.1$ is up-regulated in inflammatory $CD4^+$ T (Th1) cells of inflammatory bowel disease (IBD) model mice and that $K_{2P}5.1$ is a possible therapeutic target for IBD. However, it remains unclear the mechanisms underlying up-regulation of $K_{2P}5.1$ in Th1 cells. We recently showed that Ca^{2+}-activated K^+ channel $K_{Ca}3.1$ was post-transcriptionally regulated by histone deacetylases (HDACs) in Th1 cells of IBD model mice. Present study showed no changes in the expression levels of $K_{2P}5.1$ by HDAC inhibitor treatment in $CD4^+$ cells. Hypoxia underlies the polarization of the Th1 lymphocytes in inflamed tissues. Up-regulation of hypoxia-inducible factor (HIF)-1α was found in $CD4^+$ T cells of IBD model mice. Hypoxia (1.5% O_2) for 24 hr increased the expression levels of $K_{2P}5.1$ in mice $CD4^+$ thymocytes. These results suggest that $K_{2P}5.1$ may be up-regulated in Th1 cells of IBD model through HIF-1α-mediated signaling pathway.

2-YIA-13

Vasopressin suppresses mutual interaction between cytoplasmic tails of V1a receptors

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Agonist induces substantial changes in a cytoplasmic tail of G-protein coupled receptors. However, structural consequences of such changes have not been detected in V1a receptors, which was stimulated by full agonist. Here, we explored receptor-receptor interaction by using NanoBit split luciferase system. One of two parts of split luciferase were connected to the carboxy-terminal tail of the V1a receptor. These modifications of V1a tails did not alter vasopressin-mediated intracellular calcium signals. When V1a receptors were connected with small or large fragment of nanoluciferase and were co-expressed, strong luminescent signal was detected, indicating that V1a receptor tails formed full nanolucuferase molecule. We found that signal intensities of luciferase light gradually increased during measurement period of five minutes in human embryonic kidney cells after substrate additions. Vasopressin significantly reduced the luciferase signal intensity during the measurement period. Our data indicate that split nanoluciferase system is useful to detect small conformational changes in the cytoplasmic tails of the agonist-stimulated V1a receptor homodimers.

2-YIA-14

PEPT1-targeted boron delivery for boron neutron capture therapy using BPA-containing dipeptide

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Background: Boron neutron capture therapy is a radiotherapy utilizing the neutron capture reaction. The commonly used boron agent, *p*-borono-l-phenylalanine (BPA), is accumulated in tumors by amino acid transporters upregulated in tumors. In this study, to propose a novel strategy of selective boron delivery targeting peptide transporter, we developed BPA-containing dipeptides (BPA-Tyr and Tyr-BPA) and examined their interaction with peptide transporters and their uptake into tumor cells. **Methods**: We established HEK293 cells stably expressing PEPT1 or PEPT2, and examined their interaction with BPA-Tyr and Tyr-BPA. Dipeptide transport activity was compared among tumors with varied PEPT1 and PEPT2 expression levels. We evaluated the boron accumulation in tumors after the treatment of BPA-Tyr and Tyr-BPA *in vitro* and *in vivo*. **Results**: BPA-Tyr and Tyr-BPA are transported by PEPT1 and PEPT2. The peptide transport activity in tumor cells correlated with PEPT1 expression level. BPA-Tyr and Tyr-BPA were delivered into PEPT1-expressing tumor cells via a PEPT1-mediated mechanism *in vitro*. *In vivo*, intravenous administration of BPA-Tyr resulted in a higher accumulation in the tumors compared with the blood. **Conclusion:** PEPT1 is a promising target for cancer-specific boron delivery in BNCT, using BPA-containing dipeptide-based boron agents.

2-YIA-15

RS9, an Nrf2 activator, accelerates LC3-assosciated phagocytosis via dual activation of AMPK/mTOR and Nrf2/SQSTM1 signaling in retinal pigment epithelium

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Retinal pigment epithelium (RPE) has an important role for maintenance of visual function by phagocytosis of photoreceptor outer segment. Recent studies have elucidated that this phagocytosis shares a non-canonical form of autophagic degradation. Also, patients with non-exudative age-related macular degeneration show dysregulation of autophagic degradation in RPE. To find effective therapeutic agent, we used *in vitro* photoreceptor outer segment phagocytosis assay by RPE cells. We found that an Nrf2 activating triterpenoid, RS9, accelerated photoreceptor outer segment phagocytosis. Notably, RS9 activated both AMPK/mTOR and Nrf2/SQSTM1 signaling pathway. These signals are known to facilitate autophagic degradation by different mechanism. In the early phase, RS9 phosphorylated AMPK, then dephosphorylated mTOR. In the late phase, RS9 activated Nrf2, then induced SQSTM1. A canonical Nrf2 activator diethyl maleate showed same effects; however, it has a much weaker potential compared with RS9. In conclusion, these findings indicate that RS9 is a prominent autophagy inducer and promising therapeutic agent against non-exudative age-related macular degeneration.

2-YIA-16
Acetylcholine signaling in striatum/nucleus accumbens

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Acetylcholine is a critical neuromodulator for aversive learning and cognitive memory. For its association with Alzheimer's disease, it has drawn much of attention as the therapeutic target. The therapeutic drug improves learning and memory deficits in Alzheimer's disease, but unable to cure. Muscarinic receptor M1 (M1R) has been implicated in aversive learning and cognitive memory through its downstream mediator protein kinase C (PKC) activation. However, the mode of action of acetylcholine in neurons has not been clarified yet, as PKC signaling pathway remains unknown. Based on that acetylcholine level is the highest in striatum/nucleus accumbens (NAc) where it impacts emotional behavior, in this study we aimed to clarify the M1R-PKC pathway in striatum/NAc leading to aversive learning. By exploiting our phosphoproteomics system for comprehensive PKC substrate screening, we found that acetylcholine activates Rho family GTPase Rac and its downstream effector PAK kinase. Also, we found that the activated Rac-PAK signaling by acetylcholine in striatopallidal medium spiny neuron contributes to aversive learning. This study would advance our understanding of the mode of action of Alzheimer's disease therapeutic drugs, shedding light to the molecular basis of Alzheimer's disease.

2-YIA-17

An optogenetic approach to identify L-DOPA as a neurotransmitter in the nucleus tractus solitarii

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We have proposed that L-DOPA is a neurotransmitter. L-DOPA when exogenously microinjected into the nucleus tractus solitarii (NTS) induces depressor and bradycardic response. Electro-stimulation of aortic depressor nerves (ADN) induces L-DOPA release from the NTS, and induces depressor and bradycardic response. These our findings suggested that L-DOPA may play a neurotransmitter role in the primary baroreceptor afferents terminating in the NTS. To further determine the role of L-DOPA as a neurotransmitter in the ADN, we investigated whether depressor and bradycardic responses to L-DOPA was mimicked by stimulating ADN using optogenetic procedure in rats. We injected adeno-associated virus encoding ChR2-EYFP or EYFP into the ganglion of ADN. Four to five weeks after injection, ChR2-EYFP and EYFP signals were partially localized with tyrosine hydroxylase-positive neurons in the NTS of the fixed brain tissues. Photostimulation (473 nm, 40 mW, 20 Hz, 20s) from the surface of the NTS induces depressor and bradycardic response in the animals expressing ChR2-EYFP, but not EYFP in the ADN. The effects of photostimulation were attenuated by treatment with L-DOPA cyclohexylester (1 μg), an antagonist for L-DOPA, in the NTS, as were those of exogenously applied L-DOPA. These results suggested that L-DOPA is a neurotransmitter in the ADN terminating into the NTS.

2-YIA-18

The role of noradrenaline in the model of stress-induced seizures

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Stress is one of the most frequently self-reported precipitants for seizure induction in epilepsy patients, but how stress triggers seizures remains unknown. In the medial prefrontal cortex (mPFC), stress has been known to enhance the release of noradrenaline (NA), which excites mPFC layer 5 (L5) pyramidal cells. Thus, we investigated the possible contribution of NA in the mPFC to stress-induced epileptic seizures. Intra-mPFC infusion of picrotoxin (0.1 nmol/side) and NA (10 nmol/side) induced seizures with shorter latency than that of picrotoxin alone in C57BL/6J mice. *In vitro* whole-cell patch-clamp recordings from mPFC L5 pyramidal cells revealed that, in the presence of picrotoxin (30 μM), bath-application of NA (10 μM) induced rhythmic and frequent epileptiform activities (EA) consisting of prolonged depolarization with burst firings in short latency, while picrotoxin alone induced sporadic and long-latency EA. The NA-induced EA were inhibited by terazosin (5 μM), but not atipamezole (3 μM) or timolol (10 μM), indicating the involvement of α1 adrenoceptors for the EA generation. These results suggest that NA released in the mPFC might contribute to the expression of stress-induced seizures in epilepsy patients.

2-YIA-19

Methamphetamine-induced CPP inhibition by overexpression of Shati/Nat8l in the medial prefrontal cortex.

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Shati/Nat8l (Shati) is a novel *N*-acetyltransferase identified in the brain of mice treated with methamphetamine (METH). Recent studies showed that Shati overexpression in the nucleus accumbens (NAc) attenuates pharmacological response of methamphetamine (METH) via mGluR3. Meanwhile, it is reported that after METH self-administration and during reinstatement, dopamine (DA) and glutamate dysregulations were observed in medial PFC (mPFC) and NAc of rats. However, the regulatory mechanism of control over-reward system and the function of Shati in mPFC have not been clarified.

In this study, we injected AAV-Shati vector into the mPFC of mice. Interestingly, Shati injected mice attenuated METH-induced conditioned place preference (CPP) but not locomotor activity. Additionally, immunohistochemistry of mice injected with GFP in mPFC showed an mPFC-NAc top-down regulation. Finally, in vivo microdialysis experiments showed a decrease in DA baseline and METH-induced increased DA in the NAc. Moreover, a decrease in extracellular glutamate levels was also observed in the NAc.

These results suggest that Shati overexpression in mPFC attenuates METH-induced CPP by decreasing extracellular DA in the NAc.

2-YIA-20

Blockade of endothelin ET_B receptor ameliorates brain edema by regulation of astrocyte-derived factors after traumatic brain injury in mice

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Brain edema is a critical condition resulted from blood-brain barrier (BBB) disruption after traumatic brain injury (TBI). Several astrocyte-derived factors are involved in BBB function. We previously confirmed the involvements of endothelin ET_B receptor for BBB disruption. In this study, the effects of BQ788, an ET_B receptor antagonist on brain edema and astrocyte-derived factors were examined. As a model of TBI, fluid percussion injury (FPI) was performed on mouse cerebrum. BBB disruption and brain edema were evaluated by Evans blue extravasation and brain water content, respectively. Expressions of astrocyte-derived factors were confirmed by Real-time PCR and immunohistochemistry. To confirm therapeutic efficiencies, intraventricular (i.c.v., 15 nmol/day) or intravenous (i.v., 5 mg/kg/day) administration of BQ788 were performed from 2 to 5 days after FPI. BQ788 ameliorated FPI-induced BBB disruption and brain edema but not affected blood pressure. ET_B receptors were mainly distributed in astrocytes and BQ788 appropriately controlled expressions of several astrocyte-derived factors after FPI. Thus, blockade of ET_B receptor is expected to be a novel therapeutic strategy for brain edema.

2-YIA-21

Pathophysiological role of mitochondria-actin interactions in mouse hearts after myocardial infarction

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Defective mitochondrial dynamics through aberrant interactions between mitochondria and actin cytoskeleton is increasingly recognized as a key determinant of cardiac fragility after myocardial infarction (MI). Dynamin-related protein 1 (Drp1), a mitochondrial fission–accelerating factor, is activated locally at the fission site through interactions with actin. Here, we report that the actin-binding protein filamin A acted as a guanine nucleotide exchange factor for Drp1 and mediated mitochondrial fission–associated myocardial senescence in mice after MI.Pharmacological perturbation of the Drp1–filamin A interaction by cilnidipine suppressed mitochondrial hyperfission–associated myocardial senescence and heart failure after MI. These data demonstrate that Drp1 association with filamin, and the actin cytoskeleton, contributes to cardiac fragility after MI and suggests a potential repurposing of cilnidipine.

2-YIA-22

Involvement of TRPC3 channels in the atrial remodelings caused by chronic volume overload in rats

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Chronic volume overload to the heart has been suggested to generate arrhythmic substrates in the atria, leading to the onset of atrial fibrillation (AF). We investigated a role of TRPC channels in the pathophysiological process of atrial remodelings caused by aorto-venocaval shunt (AVS) in rats. More than 3 months after the surgery, the atrial weight of the AVS group (n = 10) was greater than that in the Sham group (n = 9), and fibrosis was observed in the atrial tissue. The P-wave duration, PR interval and QRS width of the ECG were significantly prolonged in the AVS group compared with those in the Sham group. The duration of AF induced by burst pacing in the AVS group was about 4 times longer than that in the Sham group. The mRNA levels of TRPC channel in the AVS group were significantly greater than those in the Sham group. Chronic administration of a TRPC 3 channel inhibitor pyrazole-3 to the AVS rats ameliorated the prolongation of the P-wave duration and QRS width caused by AVS surgery. Furthermore, the duration of AF was significantly shortened by the drug to the same level as the Sham group. These results suggest that TRPC3 channel is involved in the atrial remodeling caused by chronic volume overload.

2-YIA-23

Identification of a novel TRPC3-Nox2 complex inhibitor that attenuates anthracycline-induced cytotoxicity

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Doxorubicin (Dox) is a highly effective anticancer agent, but eventually induces cardiotoxicity associated with increased production of reactive oxygen species (ROS). We previously reported that the formation of protein complex between transient receptor potential canonical 3 (TRPC3) and NADPH oxidase 2 (Nox2) mediates Dox-induced cardiac atrophy in mice. The aim of this study was to identify a drug already approved for clinical use that blocks Dox-induced cytotoxicity, and demonstrate whether inhibition of TRPC3-Nox2 complex by this drug attenuates Dox-induced systemic tissue wasting in mice. Drug screening was performed using Raw264.7 macrophage cell line. We investigated whether inhibition of TRPC3-Nox2 complex contributed to attenuation of Dox-induced cytotoxicity, by measuring Nox2 protein stability through interaction between TRPC3 and Nox2. We found that ibudilast, an anti-asthmatic drug, attenuated the cytotoxicity induced by Dox and cisplatin, by inhibiting TRPC3-Nox2 functional interaction without reducing TRPC3 channel activity. These results identify a common mechanism underlying induction of systemic tissue wasting by Dox and a drug that could be repurposed to reduce the risk of cardiotoxicity by anti-cancer drugs.

2-YIA-24

Modeling Anderson-Fabry Disease with Human iPSCs Reveals Pathogenic Glycosphingolipid-Induced Cardiac Abnormalities

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Anderson **Fabry disease** (AFD) is caused by mutations in the X-linked gene ***GLA***, encoding lysosomal enzyme α-galactosidase A (**α-GAL**). The gene mutations reduce the enzymatic activity, resulting in significant lysosomal accumulation of enzyme substrates glycosphingolipids, mainly globotriaosylceramide (**Gb3**) and globotriaosylsphingosine (**lyso-Gb3**). However, how the accumulated glycosphingolipids cause disease phenotypes remain largely unknown. Here, we employed human induced pluripotent stem cells (**iPSCs**) modified with clustered regularly interspaced short palindromic repeats interference (**CRISPRi**) to model cardiac AFD *in vitro*. We found that clumps of iPS-derived cardiomyocytes (**iPS-CMs**) with CRISPRi-mediated *GLA* knockdown exhibited impaired beating, prolonged Ca^{2+} decay and relaxation. Mechanistically, α-GAL reduction increased unphosphorylated PLN by perturbing mTOR (specifically mTORC2, but not mTORC1)-AKT signaling. These results reveal how the pathogenic glycosphingolipids intersect with the **mTORC2-AKT-PLN** axis regulating relaxation and impair it, leading to the cardiac abnormalities. Our study reinforces the usefulness of the CRISPRi iPSCs in modeling monogenic diseases and delineating mechanisms of the diseases.

2-YIA-25

Thoracic duct function was impaired in spontaneously hypertensive rat

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The lymphatic system is involved in pathogenesis of edema, inflammation and cancer metastasis. It has been reported that lymph capillary network regulates interstitial electrolyte and volume balance, which buffers blood pressure. Thus, we hypothesized that impaired lymphatics dysfunction leads to increasing blood pressure. In this study, we employed thoracic duct from 10-14-week-old Wister-Kyoto (WKY) and spontaneously hypertensive rats (SHR) and examined lymphatics contractility. Thoracic duct from SHR exhibited impaired acetylcholine (ACh)-induced endothelial-dependent relaxation compared to age-matched WKY (39.4±1.0% vs 84.8±2.2%, $p<0.05$). L-NAME, a NOS inhibitor blunted ACh-induced relaxation in WKY and SHR. Tempol, a superoxide dismutase mimetic significantly improved the impairment in SHR ($p<0.05$). Thoracic duct from SHR also displayed decreased SNP-induced relaxation ($p<0.05$). Despite the marked impairment in relaxation, contraction response to angiotensin II in SHR was similar to WKY. We conclude that lymphatic function was significantly blunted in hypertensive model at least SHR through superoxide. The impairment of the lymphatic relaxation may be involved in increased blood pressure in hypertension due to dysfunction of lymphatic buffering.

2-YIA-26

Depletion of choresterol lipid efflux pump ABCG1 triggers accumulation of exosomes and regression of tumors

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The ATP-binding cassette transporter G1 (ABCG1) is a cholesterol lipid efflux pump whose role in tumor growth has been largely unknown. Our transcriptomics revealed that ABCG1 was powerfully expressed in rapidly metastatic, aggregative colon cancer cells, in all the ABC transporter family members. Coincidently, genetic amplification of *ABCG1* is found in 10% to 35% of clinical samples of metastatic cancer cases. Expression of ABCG1 was further elevated in three-dimensional tumoroids (tumor organoids) within stemness-enhancing tumor milieu, whereas depletion of ABCG1 lowered cellular aggregation and tumoroid growth in vitro as well as hypoxia-inducible factor 1α in cancer cells around the central necrotic areas in tumors in vivo. Notably, depletion of ABCG1 triggered the intracellular accumulation of extracellular vesicles (EVs) and regression of tumoroids. Collectively, these data suggest that ABCG1 plays a crucial role in tumorigenesis in metastatic cancer and that depletion of ABCG1 triggers tumor regression with the accumulation of EVs, their derivatives and cargos, implicating a novel ABCG1-targeting therapeutic strategy by which redundant and toxic substances may be accumulated in tumors leading to their regression.

2-YIA-27

Suppression of tumor angiogenesis by inhibition of LAT1 in stromal endothelial cells

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Angiogenesis, a process of vascular growth from preexisting vessels, is involved in pathological processes including tumor growth and metastasis. L-type amino acid transporter 1 (LAT1) is highly expressed in a wide range of cancers and contributes to supplying amino acids. LAT1 has, thus, been proposed as a potential target for cancer therapy. Recently, we found that LAT1 shows high expression not only in tumor cells but also in endothelial cells of pancreatic cancer tissues. In contrast, the expression of LAT1 in endothelial cells of normal tissues is limited. In this study, we examined whether LAT1 is involved in tumor angiogenesis or not. LAT1 inhibitors reduced the number of blood vessel sprouting in *ex vivo* aortic ring assay. Suppression of angiogenesis by LAT1 inhibitors was further confirmed in *in vivo* matrigel plug assay and xenograft tumor model. Moreover, contribution of LAT1 in angiogenesis was verified using human umbilical vein endothelial cells in *in vitro* angiogenesis assays. LAT1 inhibitors are supposed to exert an anti-angiogenic effect through the inhibition of LAT1 in the endothelial cells of tumors, which could, together with the suppression of amino acid uptake of cancer cells, contribute to the anti-tumor effect *in vivo*.

2-YIA-28

Explore the effect of PHD inhibitor on innate immune systems in tumor mouse model.

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Tumor microenvironment (TME) is hypoxic and low pH. TME confer not only resistance of anti-cancer drug therapy on tumor cells, but also impair the activity of immune cells. Under TME condition, tumor-infiltrated macrophages (Mfs) change phenotype from anti-tumor (M1) phenotype to tumor progressive (M2) phenotype. Previously we reported that prolyl hydroxylase (PHD) inhibitor, which activate hypoxia-induced factors (HIFs), improve TME. In this study, we examined whether the improvement of TME by PHD-inhibitor activate tumor-infiltrated Mfs. Lewis lung carcinoma cells were subcutaneously transplanted into right frank of mice which aged at 8-12 weeks. Mice were treated with PHD inhibitors intraperitoneally at day 10 after tumor transplantation. Then tumor tissues were collected at day 16 and analyzed immune cells by immunofluorescence staining and flowcytometry. We performed phagocytosis assay using sorted Mfs from tumor tissue and bone derived Mfs. Furthermore, we injected sorted Mfs into the tumor and evaluated tumor growth. PHD-inhibitor inhibited tumor growth. We concluded that PHD inhibitor directly activated Mfs and TME improvement supported activity of Mfs.

2-YIA-29

GRK2 upregulates iNOS expression in microglia by mediating IRF1 and STAT1/3 activation

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G protein-coupled receptor (GPCR) kinase 2 (GRK2) has emerged as an integrating node in many signaling pathways besides its GPCR signaling role. We investigated how GRK2 could contribute to Toll-like receptor (TLR4) signaling for iNOS expression in microglia. We found in microglial cells LPS-induced iNOS expression requires both STAT1 and STAT3 activation. GRK2 knockdown by siRNA transfection suppressed phosphorylation and nuclear translocation of STAT1/3. Interferon-β (IFN-β) produced from TLR4 signaling induce phosphorylation of STAT1/3, and its expression is regulated by interferon regulatory factors (IRF) at transcription level. We found that GRK2 knockdown suppressed LPS-induced IFN-β production and mRNA expression. LPS stimulation induce IRF1 expression and nuclear translocation, and GRK2 knockdown suppressed IRF1 nuclear translocation without affecting expression level. These results suggest that GRK2 positively regulates IRF1 activation to induce IFN-β expression, STAT1/3 activation, and subsequently iNOS expression. Furthermore, GRK2 knockdown suppressed exogenous IFN-β-induced STAT1/3 activation. In conclusion, GRK2 upregulates TLR4-induced iNOS expression by mediating IRF1 and STAT1/3 activation.

2-YIA-30

Dynamic changes in host genome 3D structure in response to influenza virus infection

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Genome is spatially organized in the nucleus. The chromatinized DNA segregates into an active "A" compartment, which contains gene-dense chromatin bearing active epigenetic marks, and an inactive "B" compartment, which contains gene-poor chromatin bearing repressive epigenetic marks. Compartments represent the long-distance interaction patterns including topologically associating domains (TADs) and lamina-associated domains (LADs). Influenza virus replicates its genome in the nucleus and viral proteins have shown to interact with multiple components of the host epigenetic machinery to promote viral replication and limit host immune responses. However, it is unknown how influenza virus infection can affect the spatial configuration of chromatin and function of the host genome in the nucleus. In the present study, using genome-wide chromosome conformation capture (Hi-C) technique we showed the dynamic changes in TADs and A/B formations during the course of influenza virus infection in mouse embryonic fibroblasts. In addition to nuclear lamina disruption, DamID-seq demonstrated the changes in LADs formation following virus infection. Thus, our data suggest dynamic changes in host genome 3D structures to virus infection, which could be a novel therapeutic target for influenza virus infection.