2-S18-1 Symposium18

Iron metabolism disturbance induced by repeated social defeat stress

Shiho Kitaoka

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Acute stress activates sympathetic nervous system and endocrine system as a survival mechanism. However, prolonged or excess stress induces behavioral abnormalities and cognitive dysfunction.

We previously reported that repeated stress activates microglia via Toll-like receptor 2 and 4 to produce proinflammatory cytokines such as IL-1a and TNFa in the medial prefrontal cortex, thereby leading to the induction of social avoidance. These results demonstrate that repeated stress induces inflammation-like response in the brain to induce behavioral changes. We also found that repeated stress promotes leukocytes infiltration to the brain parenchyma, suggesting the interaction between brain and peripheral immune systems.

As it is well-known that repeated stress is a risk factor not only for mental illnesses but also for cardiovascular disease and metabolic diseases, repeated stress perturbs the function of peripheral organs. Since repeated stress alters immune system through the activation of sympathetic nervous system, we analyzed peripheral blood. We found that repeated stress induces iron-deficiency anemia. In this symposium, I will introduce my recent work on repeated stress-induced disturbances in iron metabolism and discuss its behavioral relevance.

2-S18-2 Symposium18

Involvement of gut microbiome in the behavioral abnormality by early weaning

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Mammalian infants heavily depend on their mothers, and mother-infant interactions greatly influence neurobehavioral development. We have shown that early weaning consistently affect the emotional development in mice. Early-weaned mice show increased anxiety-like behaviors, heightened hypothalamic–pituitary–adrenal axis activity, and prolonged reduction of the expression of brain-derived neurotrophic factor (BDNF) in the prefrontal cortex (PFC). Recently, we found that normalizing circulating corticosterone in early-weaned mice, either in adulthood or soon after weaning, ameliorated anxiety levels. Anxiety in early-weaned mice was also ameliorated by pretreatment with glucocorticoid receptor antagonist or BDNF into the PFC. These suggest that early weaning increased anxiety levels by modulating glucocorticoid and BDNF signaling in the PFC. As other mechanisms, we are now focusing on the gut microbiome. It has suggested that the gut microbiome does not only affect intestinal cells locally, but also impact the central nervous system. In this symposium, we would like to discuss our current study evaluating the developmental impact of the microbiome by analyzing offspring of germ-free mother mice orally administrated the fecal microbiome of early-weaned mice.

Anlaysis of multiple organ interactions by recordings and manipulations of vagus nerve spiking activity

Takuya Sasaki

Lab. Chem. Pharmacol., Grad. Sch. Pharm., Tokyo Univ.

The vagus nerve serves as a central pathway for communication between the central and peripheral organs. Despite traditional knowledge of vagus nerve functions, detailed neurophysiological dynamics of the vagus nerve in naïve behavior remain to be understood. In this study, we developed a new method to record spiking patterns from the cervical vagus nerve while simultaneously monitoring central and peripheral organ bioelectrical signals in a freely moving rat. When the rats transiently elevated locomotor activity, the frequency of vagus nerve spikes was correspondingly increased, and this activity was retained for several seconds after the increase in running speed terminated. During stopping, the vagus nerve spike patterns differed considerably depending on external contexts and peripheral activity states associated with cortical arousal levels. These observations are a new step for uncovering the physiological dynamics of the vagus nerve modulating the visceral organs such as cardiovascular, respiratory, and gastrointestinal systems. Moreover, I will present some preliminary data including a new method to operate the spiking activity of vagal fibers using transgenic mouse lines, which will be useful for further understanding central-peripheral organ interactions.

Vagal nerve signal-regulated cell proliferation for maintaining whole body homeostasis

Junta Imai, Hideki Katagiri

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When organs are damaged, cells proliferate to repair these organs. On the other hand, pancreatic β-cells adaptively proliferate in insulin-resistant states to increase insulin production. Therefore, these proliferations are compensatory mechanisms aimed at maintaining whole body homeostasis and survival. We previously discovered an inter-organ neuronal relay system, consisting of the afferent splanchnic nerve, central nervous system and efferent vagus, which is involved in adaptive β-cell proliferation. We elucidated the underlying molecular mechanisms which involve neurotransmitters from the vagus and activation of the β-cell FoxM1 pathway. We also recently found that vagal signals activate the hepatic FoxM1 pathway, thereby regulating acute liver regeneration after hepatic injury and that this system is critical for supporting survival. Therefore, vagal signal-regulated cell proliferation is involved in tissue adaptation in response to increased insulin demand and tissue repair after severe organ damage in β-cells and the liver, respectively.

These results enhance our understanding of adaptation and recovery systems of organs/tissues as well as clarifying the pathogenesis of several diseases attributable to impaired adaptive tissue proliferation. Furthermore, our results may well provide novel clues for developing tissue regeneration strategies based on endogenous biological systems.

Development of a novel peroxisome proliferator-activated receptor alpha activator to treat nonalcoholic steatohepatitis

Keisuke Tachibana, Takefumi Doi

Grad. Sch. Pharm. Sci., Osaka Univ.

Nonalcoholic steatohepatitis (NASH), which is characterized by triglyceride accumulation in hepatocytes, causes liver fibrosis and cancer. Peroxisome proliferator-activated receptor alpha (PPARα), a ligand-activated transcription factor, is predominantly expressed in the liver, where it activates fatty acid catabolism and reduces plasma triglyceride levels. Recent studies suggest that PPARα is a good therapeutic target for NASH. In this study, to detect PPARα activators, we established reporter cell lines to quantify the effects of ligands on PPARα activity using a tightly tetracycline-regulated human hepatoblastoma cell line that can be induced to express full-length human PPARα. By screening a chemical library using this cell line, we successfully identified 1*H*-pyrazolo-[3,4-*b*]pyridine-4-carboxylic acid derivatives as hit compounds with different basic skeletons from those of known PPARα agonists. This compound upregulated PPARα transcriptional activity in a dose-dependent manner, and induced PPARα target genes both in vitro and in vivo. Treatment of NASH model mice with this compound via oral gavage for 6 weeks led to a reduction in the plasma triglyceride level and a slight decrease in the liver hydroxyproline content. We are currently conducting X-ray crystallographic studies of the PPARα ligand-binding domain and complexes of these compounds to design and develop more effective drugs. Although further investigations are needed, this novel PPARα ligand might be a candidate drug for treating NASH.

Application for drug discovery using mammalian artificial chromosomes

Yasuhiro Kazuki

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The conventional vector system for expression of desired genes in mammalian cells has problems such as insertion of the vector into the host and restriction of the introduced DNA size. To solve the problems, we constructed a human artificial chromosome (HAC) vector and a mouse artificial chromosome (MAC) vector that do not contain any endogenous genes using our unique chromosome engineering technology. The HAC/MAC as gene delivery vectors can deliver Mb-sized gene cluster and multiple genes, and are stably and independently maintained with defined copy numbers in host cells, as well as being transferrable to any other cell line via microcell-mediated chromosome transfer (MMCT). We have demonstrated several applications for drug discovery using the HAC/MAC, including 1) Humanized mice/rats and multifunctional model cells for predicting human drug metabolism, 2) Novel Down syndrome model animals and cells for identifying the responsible genes and the preclinical study, 3) Basic study for gene and cell therapy of Duchenne muscular dystrophy, and 4) Fully human antibody-producing animals for antibody drug. Thus, HAC/MAC technology can be expected to be used not only for basic research but also for applied research such as drug discovery and medical applications.

Cardiovascular Safety Tests for the Promotion of Drug Development in Academia

Atsuhiko Naito

Dept. Cell Phys., Grad. Sch. Med., Toho Univ.

Cardiac toxicity is the leading cause of attrition during drug development. International Consortium on Harmonization provides the guideline for evaluating the potential arrhythmogenicity of drug candidates based on *in vitro* assessment of I_{Kr} inhibition by hERG test and *in vivo* measurement of QT interval.

The purpose of this study is to support the drug development in Japanese non-profit research institutions through undertaking the cardiac safety tests that would be laborious to perform in each institution. For that purpose, we perform hERG test and *in vivo* QT assay using canines to assess the cardiovascular safety of drug candidates those developed in Japanese non-profit research institutions. Another purpose of this study is to develop the next-generation system to more accurately predict cardiovascular toxicity of drug candidates. For that purpose, we establish a novel *in vitro* assay using human induced pluripotent stem cell-derived cardiomyocytes to assess the toxicity that impair cardiac function. We also characterize *microminipigs* as the novel animal model for assessing the cardiovascular safety of drug candidates in the future world. We believe that our research would improve the quality and the safety of the drugs candidates those are developed in Japanese non-profit research institutions.

Academic drug discovery with pharmaceutical modality in Center for Research and Education on Drug Discovery of Hokkaido University

Katsumi Maenaka

Facult. Pharm. Sci., Hokkaido Univ.

Hokkaido University Institute of Pharmaceutical Sciences established an affiliated drug discovery science research and education center, Center for Research and Education on Drug Discovery of Hokkaido University (CRED), in 2011 to promote academia drug discovery research. We have set up over the last 8 years the ‘state of the art' instruments necessary for chemical screening, and provided the expertise for drug discovery to novice users, supported by the projects, such as "Basis for Supporting Innovative Drug Discovery and Life Science Research". We also organize a "Platform for medical care and drug discovery" network with related academic departments and universities, Hokkaido University hospital and pharmaceutical companies mainly in Hokkaido Area.

We are proceeding drug discovery modality research using a seamless "from drug seeds to preclinical" development system with a cryo-electron microscope: (1) unique Hokkaido University chemical library (peptides, nucleic acids and natural compounds) and semi-automatic preparation system, (2) preparation technology of difficult-to-express proteins, (3) complete physicochemical measurement technology, (4) Integrated structural analysis technology with cryo EM.

In this lecture, I would like to look back over the past eight years and introduce the current efforts of CRED with some specific examples including the cooperation with the Development Unit of Drug Discovery Initiative of the University of Tokyo to proceed ADME evaluation and derivative synthesis.

2-S20-1 Symposium20

Current status and issues in clinical use of opioids: expectations for development of novel analgesics

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In Japan, 13 opioids with a variety of dosage forms, such as oral preparation, injection, transdermal patch, transmucosal and suppository are clinically available as analgesics, and their use properly depends on the type and degree of pain and patient condition. Fundamentally, strong opioids are restricted to only treatment of cancer pain, while the indication of fentanyl transdermal patch has been expanded for the treatment of non-cancer chronic pain. In US, the additional indication of opioids to chronic pain has led to prolongation and generalization of opioid use, which may contribute to "opioid crisis" in which opioid-related death strikingly increased due to opioid abuse and overdose-induced respiratory depression. Currently, opioid-related abuse and death have not been evident in Japan, while abuse of antitussive opioids (codeine and dihydrocodeine) are recently seen as a problem, which may suggest the sense of guilt to abuse legally-uncontrolled drugs (ex. weak opioids, antitussives or hypnotics) may be low in Japanese. In this symposium, I will summarize current status and issues in clinical use of opioid analgesics, and will talk about the necessity and expectation for development of novel analgesics without serious adverse reactions such as addiction and respiratory depression.

Further classification of μ-opioid analgesics based on “intracellular biased-signaling through μ-opioid receptors”

Naoko Kuzumaki, Minoru Narita

Dept. Pharmacol., Hoshi Univ.

Since the choice of opioid analgesics that can be used for supportive and palliative care in patients with cancer has increased, it is necessary to understand the intracellular signaling of each drug. Although all opioid analgesics used in clinical practice are μ-opioid receptor agonists, they exhibit individual profiles with different levels of analgesia and side effects. Recently, it has been shown that μ-opioid receptor agonists regulate at least two pathways after the stimulation of μ-opioid receptors: a G protein-coupled signaling pathway and a β-arrestin recruitment pathway. In the present study, we further re-classified μ-opioid analgesics by organizing individual drug profiles based on intracellular responses generated by various μ-opioid analgesics. We here propose the usefulness of appropriate combinations of μ-opioid receptor agonists due to biased classification with intrinsic signaling. In this symposium, we will discuss the latest knowledge on μ-opioid receptor-biased signaling by μ-opioid analgesics.

MOPr-DOPr heteromer: The meaning and possibility as novel therapeutic target for pain control

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Many studies suggest opioid receptor (OPr) dimerization modulates the pharmacological properties of opiates. Specifically, heteromerization between OPr types has been reported to lead to changes in intracellular signaling. Thus, ligands targeting heteromers are expected to be novel therapeutic targets with reduced side effects. The heteromers of mu (MOPr) and delta (DOPr) are detected in brain regions involved in pain processing. By high-through-put screening, CYM51010 was identified as a MOPr-DOPr-biased ligand. Furthermore, CYM51010 exhibits antinociceptive properties similar to that of morphine with lesser antinociceptive tolerance as compared to morphine. Studies exploring the *in vivo* regulation of MOPr-DOPr heteromers, showed chronic morphine administration leads to an upregulation of these heteromers in select brain regions. Exploration of mechanisms underlying this phenomenon led us to the G protein-coupled receptor chaperone, RTP4, that is induced by chronic morphine and facilitates the heteromerization of MOPr and DOPr. In this presentation, I will present the identification and characterization of CYM51010 and the role of RTP4 in heteromer regulation that could serve as a scaffold for the development of novel therapeutic drugs with reduced adverse effects, and hence may take place of the conventional clinical opioids.

2-S20-4 Symposium20

Safe opioid analgesics targeting nociceptin receptor in primates

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Although mu opioid peptide (MOP) receptor agonists are widely used as standard analgesics, the abuse potential and some adverse effects have contributed to escalating medical and economic burdens in the global community. Given that the nociceptin/orphanin FQ (N/OFQ) peptide (NOP) receptor agonists exert antinociception without producing nonpreferable effects at analgesic doses in nonhuman primates, we hypothesized that bifunctional NOP/MOP agonists may be a viable approach to generate safer opioid analgesics.

Newly developed bifunctional NOP/MOP receptor agonists, which shows partial agonist activity for both NOP and MOP receptors, exerted morphine-comparable antinociception without causing itch sensation following systemic or intrathecal administration in rhesus monkeys. Unlike other MOP receptor agonists, bifunctional NOP/MOP receptor agonists did not show adverse effects commonly associated with MOP receptor agonists (i.e., respiratory depression, abuse potential, opioid-induced hyperalgesia, and physical dependence).

Our findings in nonhuman primates suggest that bifunctional NOP/MOP agonists with the appropriate balance of NOP and MOP agonist activity, may provide innovative safer opioid analgesics that beget ideal pain control.

2-S21-1 Symposium21

The role of primary cilium in cell growth and differentiation

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Primary cilia are microtubule-based sensory organelles that organize numerous key signaling during development and tissue homeostasis. Defects in primary cilia formation and dysregulated ciliary functions result in multiple genetic diseases, such as obesity, polycystic kidney and tumorigenesis. We have previously shown that a centriolar protein trichoplein functions as a negative regulator of ciliogenesis by activating Aurora A kinase. In proliferating cells, trichoplein is stabilized by USP8 deubiquitinase that is phosphorylated and activated by EGFR kinase. When cells are exposed to cell cycle arrest signals, such as serum starvation, the EGFR-USP8 pathway is down-regulated, and thereby $CRL3^{KCTD17}$ ubiquitin E3 ligase induces trichoplein degradation and ciliogenesis. Recently, we found that trichoplein knockout mice were obesity-resistant, and high-fat diet consumption had little effects on body weight. The mice have long cilia in various tissues and restrict both the high-fat diet- and the injury-mediated adipogenic differentiation. Moreover, trichoplein knockdown suppresses adipogenic differentiation of murine mesenchymal-like C3H10T1/2 cells through the long cilia-mediated inhibition of insulin signal. In this session, I would like to talk about the roles of primary cilia in cell growth and differentiation.

An investigation of the role of the ubiquitin-proteasome system using zebrafish

Yuhei Nishimura

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The ubiquitin-proteasome system regulates a wide range of cellular processes, including proliferation, differentiation, and apoptosis. Primary cilia, nonmotile antenna-like structures observed in a variety of vertebrate cells, detect extracellular cues and transduce these signals into cells to regulate proliferation and differentiation. It has been known that the ubiquitin-proteasome system regulates assembly and disassembly of primary cilia. However, the molecular mechanisms underlying the regulation remain incompletely understood. We have revealed that trichoplein, a centriolar protein originally identified as a keratin-binding protein, suppresses ciliogenesis through the activation of Aurora A kinase. We have also revealed that trichoplein is ubiquitinated by CRL3-KCTD17 and deubiquitinated by USP8. To analyze the functions of trichoplein, KCTD17, and USP8 in vivo, we have generated knockout zebrafish lines for these genes. In this symposium, I would like to discuss the phenotypes of these zebrafish lines and the involvement of the ubiquitin-proteasome system and the primary cilia in the phenotypes.

Drug discovery of small molecule modulators for ubiquitin-proteasome system with *in silico* screening strategy, INTENDD

Hirotsugu Komatsu, Ken Ikeda, Takeshi Tanaka, Takao Matsuzaki

Interprotein Corporation

It is often mentioned that drug targets have been exhausted. However, large number of ubiquitin-proteasome system-related targets have not been examined yet and have a great potential as reservoir of drug targets. Although hit identification of protein degradation inhibitors and inducers including ubiquitin-proteasome system-targeting compounds are usually conducted by high throughput screening (HTS), literature-based compound synthesis evolvement or binding energy-based standard *in-silico* screening, we have experienced that it is hard to identify good hit compounds for protein degradation inhibitors and inducers by these approaches. Based on these situations, Interprotein established INTerprotein's Engine for New Drug Design (INTENDD), a proprietary *in-silico* screening strategy that propose hit candidates by "binding mechanism"-based algorithm but not binding energy-based selection for final identification of hit candidates. Furthermore, utilizing INTENDD's knowhows, we also constructed AI-guided INTENDD, an artificial intelligence (AI)-introduced activity prediction system that is expected to accelerate lead generation/optimization of small molecules. In my presentation, I will introduce advantages of INTENDD and AI-guided INTENDD, and those applications for challenging drug targets including ubiquitin-proteasome system.

2-S21-4 Symposium21

Ubiquitin-proteasome drug development with SNIPER technology

Mikihiko Naito

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Development of potent inhibitors against disease-related proteins, such as oncogenic kinases, is a promising approach for novel drug discovery. However, it is hard to develop effective inhibitors against intracellular proteins without enzymatic activity. These proteins are sometimes called "undruggable proteins", and account for approximately more than 70 % of the proteins expressed in cells. Inducing protein degradation by small molecules is a novel technology that can be applied to target the undruggable proteins. We and others have developed chimeric degrader molecules named PROTACs (Proteolysis-Targeting Chimeras) and SNIPERs (Specific and Nongenetic IAP-dependent Protein Erasers) that induce degradation of the target proteins in a highly selective manner. These chimeric molecules contain a target-ligand linked to a ligand for E3 ubiquitin ligases such as $CRL2^{VHL}$, $CRL4^{CRBN}$ and IAPs, and they induce poly-ubiquitylation and proteasomal degradation of the target proteins. Substitution of the target-ligand allows us to rationally design a novel degrader molecule against protein of interest. In the lecture, I would like to show data of our SNIPER compounds and discuss the future prospect of the protein degraders.

Doxorubicin induces trans-differentiation in cardiac fibroblasts via cell death-independent pathways

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Background: Doxorubicin (DOX)-induced heart failure has a poor prognosis, and effective treatments have not been established. The effect of DOX on cardiac fibroblasts at non-lethal concentrations remains unknown. The aim of this study was to investigate the direct effect of doxorubicin on the activation of cardiac fibroblasts independent of cell death pathways. **Methods:** An animal study was performed to confirm the effects of a lower dose of DOX than a toxic cumulative dose. Human cardiac fibroblasts (HCFs) were used. The effects of DOX on mRNA expression were evaluated by microarray analysis. mRNA of collagen and the other fibrotic factor were measured by RT-PCR. Protein expression was evaluated by western blot. **Results:** DOX-induced fibrosis was localized to the perivascular area in mice. Microarray analysis showed that DOX increased the expression of the immune system, inflammatory reaction and matrix metalloproteinase (MMP) genes, resulting in cardiac remodelling. DOX enhanced mRNA of alpha smooth muscle actin (α-SMA) (a marker of trans-differentiation), interleukin (IL)-1, IL-6, transforming growth factor (TGF)-β, collagen and MMP1 expression in less than 0.1 μM which did not inhibit the cell viability in HCFs. DOX also promoted the protein expression of fibrotic markers, such as α-SMA. Furthermore, DOX induced mitochondrial damage and mitophagy. **Conclusions:** These findings suggested that doxorubicin directly induced fibrotic change of cardiac fibroblast via cell death-independent pathway. There may be potentially new mechanisms of doxorubicin induced cardiotoxicity.

The roles of gap junctional intercellular communication in non-alcoholic steatohepatitis (NASH) and liver fibrosis

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Non-alcoholic steatohepatitis (NASH) is a common risk factor for fibrosis, cirrhosis, and a predisposing factor for the development of hepatocellular carcinoma. Increase of incidence of NASH has become as a major worldwide public health problem. Connexin (Cx)32, a hepatocyte gap-junction protein, plays an important role in liver tissue homeostasis. However, the precise contribution of Cx32 in the development of NASH and fibrosis has not been established. Therefore, roles of Cx32 on NASH and fibrosis was explored by using Cx32 dominant negative transgenic (Cx32ΔTg) rat model. Extensive fibrosis was evident in Cx32ΔTg as compared to wild-type (Wt) rats; the developing fibrous septa were extended not only from the portal area to the centrilobular zone but also to adjacent portal tracts. Cirrhosis with bridging fibrosis was also recognized in some Cx32ΔTg rat livers. Elevasion of reactive oxygen species, inflammatory cytokine expressions (Tnfα, Il6, Tgfβ, Il1β, Timp2 and Col1a1), and NF-κB activity were clearly severe in Cx32ΔTg rats and these changes and fibrosis was suppressed by some anti-oxidants. These results suggest that accumulation of oxidative stress induced by Cx32 dysfunction contributes to fibrogenic remodeling in the liver. We would like to discuss about molecular mechanisms and chemoprevention of NASH and liver fibrosis.

2-S22-3 Symposium22

exploring mechanisms of renal fibrosis progression

Daisuke Nakano

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Kidney is the organ that has one of the most complicated architecture in the body. The sophisticated architecture of nephron made its physiology difficult to unravel and the variety for experimental approach for renal (patho) physiology is limited so far. At this symposium, I would like to discuss the recent progress in our understanding of renal fibrosis, including peritubular capillary flow regulation and the interaction with tubules. Renal peritubular capillary blood flow plays a role in many biological functions, including supplying oxygen to tubular and interstitial cells and recycling reabsorbed electrolytes, glucose, and amino acids. Diseased kidney often show the heterogeneity of blood flow in each capillary in several experimental models of disease; local ischemia in the capillary bed could influence neighboring tubular function, and the balance between the ischemic and normoxic capillary number correlates with renal function. We recently found that ischemia/reperfusion-induced damage changed renal tubular glucose handling and that proximal tubular glucose uptake plays important roles in the balance between recovery versus development of fibrosis after renal ischemia/reperfusion-induced injury, which can be mediated by SGLT2 inhibitors through reconstructing the renal capillary network. Another hand, from the pharmacological viewpoint, one of the biggest problem on the anti-renal fibrotic drug development is lacking the good animal model. While there are several I/R models for rodents, each model has limitation to develop broad fibrotic changes in the kidney, such as high mortality and shrinking the kidney. We recently succeeded to create an experimental model of renal fibrosis that is possible to estimate the later phase fibrotic level based on the blood urea nitrogen at day 1 of final surgery. This model makes easy to design the experimental protocol for assessing the therapeutic potential of the specific drug being developed for the purpose of treating renal fibrosis.

Vascular remodeling in pulmonary arterial hypertension: roles of CaSR and PDGF

Aya Yamamura

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Pulmonary arterial hypertension (PAH) is a progressive and fatal disease associated with remodeling of the pulmonary artery. The major pathogenesis of PAH is sustained pulmonary vasoconstriction and pulmonary vascular remodeling. Excitable and structural abnormality in the pulmonary artery, such as vasoconstriction and vascular remodeling in PAH patients, are mostly mediated by an elevated cytosolic Ca^{2+} concentration in pulmonary arterial smooth muscle cells (PASMCs). We previous found that the Ca^{2+}-sensing receptor (CaSR) is upregulated in PASMCs from idiopathic pulmonary arterial hypertension (IPAH) patients, and contributes to enhanced Ca^{2+} responses and excessive cell proliferation (vascular remodeling). In this study, the molecular mechanisms underlying the upregulation of CaSR were examined in PASMCs from normal subjects and IPAH patients. In normal-PASMCs, expression of CaSR was increased by platelet-derived growth factor (PDGF), which is known as an endogenous signal associated with IPAH. The expression of PDGF receptors was higher in IPAH-PASMCs than in normal-PASMCs. PDGF-induced activation of PDGF receptors and its downstream molecules (ERK1/2, p38, Akt, and STAT1/3) sustained longer in IPAH-PASMCs. In addition, PDGF stimulation facilitated both proliferation and migration of normal-PASMCs. On the other hand, siRNA knockdown of PDGF receptors attenuated the CaSR upregulation in IPAH-PASMCs. Imatinib (an tyrosine kinase inhibitor of PDGF receptors) and NPS2143 (an antagonist of CaSR) inhibited the PDGF-induced CaSR upregulation in IPAH-PASMCs. These results suggest that PDGF signal activates the upregulation mechanism of CaSR in IPAH-PASMCs. In conclusion, the linkage between CaSR and PDGF signals is a novel pathophysiological mechanism contributing to the development of PAH including excessive cell proliferation (vascular remodeling). The combination of tyrosine kinase inhibitors of PDGF receptors and CaSR antagonists may be useful for the treatment for PAH.

2-S23-1 Symposium23

The role of central nervous system in the regulation of glucose homeostasis

Hiroko Ikeda

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Since the disturbance of glucose metabolism causes diabetes mellitus and related diseases, it is important to regulate plasma glucose levels properly. Plasma glucose levels are regulated by the balance with glucose production and glucose utilization. It is well known that glucagon and insulin secreted by the pancreas play important roles in the regulation of plasma glucose levels. In addition, the liver regulates blood glucose levels by glycogenolysis and gluconeogenesis. These functions are controlled by autonomic nerves. In contrast, recent evidence suggested that the central nervous system (CNS) regulates blood glucose levels. It is known that the hypothalamus regulates energy homeostasis including glucose metabolism. Moreover, it is reported that the hypothalamus regulates the sympathetic and parasympathetic nerves. We have shown that an anti-psychotic drug olanzapine increases plasma glucose levels through sympathetic nerves. Thus, it is suggested that the CNS regulates plasma glucose levels through autonomic nerves. We have recently focused on central dopaminergic functions, which is one of the action sites of olanzapine, and found how central dopaminergic functions regulate blood glucose levels. In this symposium, I would like to review how the CNS regulates glucose metabolism and to introduce our recent research.

MicroRNAs 106b and 222 improve hyperglycemia in a mouse model of insulin-deficient diabetes via pancreatic β-cell proliferation

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Major symptoms of diabetes mellitus manifest, once pancreatic β-cell numbers have become inadequate. Although natural regeneration of β-cells after injury is very limited, bone marrow (BM) transplantation (BMT) promotes their regeneration through undetermined mechanism(s) involving inter-cellular (BM cell-to-β-cell) crosstalk. We found that two microRNAs (miRNAs) contribute to BMT-induced β-cell regeneration. Screening murine miRNAs in serum exosomes after BMT revealed 42 miRNAs to be increased. Two of these miRNAs (miR-106b-5p and miR-222-3p) were shown to be secreted by BM cells and increased in pancreatic islet cells after BMT. Treatment with the corresponding anti-miRNAs inhibited BMT-induced β-cell regeneration. Furthermore, intravenous administration of the corresponding miRNA mimics promoted post-injury β-cell proliferation through Cip/Kip family down-regulation, thereby ameliorating hyperglycemia in mice with insulin-deficient diabetes. Thus, these identified miRNAs may lead to the development of therapeutic strategies for diabetes.

High Phosphate diet affects the regulation of endocrine FGFs functions.

Hiroshi Kurosu

Division Anti-aging Medicine, Jichi Medical University

The endocrine FGFs require the Klotho family as co-receptors for high affinity binding to their cognate FGFRs. FGF23 requires alphaKlotho to bind to FGFRs and functions as a phosphaturic hormone and as a counter-regulatory hormone of Vitamin D_3. In response to increased dietary intake of phosphate, FGF23 is secreted from the bone. FGF23 acts on the kidney to suppress phosphate re-absorption and Vitamin D_3 synthesis. Identification of the FGF23-alphjaKlotho endocrine axis has substantially advanced our knowledge of phosphorus metabolism and transformed our view of the role of phosphate.

Recent clinical studies demonstrated that, in addition to FGF23, plasma FGF21 levels were significantly increased with the progression of early- to end-stage chronic kidney disease (CKD) . In the present study, we investigated the effect of a high phosphorus diet on the endocrine FGFs and found that the HP diet induced up-regulation of FGF23 and FGF21 mRNA expression in the bone and the liver, respectively. On the other hand, the HP diet also induced remarkable down-regulation of FGF15 mRNA expression in the ileum. FGF21 and FGF15 require betaKlotho to bind to their cognate FGFRs, FGFR1c and FGFR4, respectively. These findings indicate that phosphate overload induces the cross-talk between the FGF-Klotho endocrine axes and potentially contributes to pathophysiology of CKD.

2-S23-4 Symposium23

Regulation of glucagon secretion and pharmacotherapy for diabetes

Licht Miyamoto, Koichiro Tsuchiya

Dept. Medical Pharmacology, Inst. of Biomedical Sciences, Tokushima Univ.

Recent studies have suggested significance of glucagon in diabetes. Recent topics related to glucagon and pharmacotherapy of diabetes will be discussed. SGLT2 inhibitors lower blood glucose levels by preventing renal glucose reuptake, but they often cause hyperglucagonemia. Potential role of SGLT2 as a glucagon suppressor in pancreatic alpha cells was demonstrated recently, though it has been under debate. Thus, we conducted functional analyses of SGLT2 in a typical model of pancreatic alpha cell, α-TC cells to unveil roles of SGLT2 in the glucagon secretion. Glucagon secretion as well as intracellular ATP level decreased in response to glucose deprivation. SGLT2 inhibitors reduced glucose uptake, but glucagon secretion nor ATP level was affected. An inhibitor of KATP channel increased glucagon secretion without changing ATP level. Therefore, glucose starvation should not facilitate but suppress glucagon secretion possibly by raising AMP/ATP ratio which mitigates membrane potential through KATP channel. We also found SGLT2-mediated glucose uptake in α-TC cells. Nevertheless, the glucose influx is supposed to be too small to take effects on ATP level, and SGLT2 inhibitors should not directly alter glucagon secretion. Glucose starvation-induced glucagon secretion may require interaction among different types of the cells in islets.

2-S24-1 Symposium24

Regulation of pain signaling by the innate immune system

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The innate immune system is the body's first response to infections and its activation gives rise to pain. How the innate immune system interacts with the sensory nervous system and contributes to pain is poorly understood. We have shown previously that intraplantar CFA injection leads to an upregulation of the deubiquitinase USP5 in dorsal root ganglia and spinal cord, and this in turn leads to an increase in the numbers of Cav3.2 T-type calcium channels in an activity dependent manner. Blocking USP5 interactions with cell permeant disruptor peptides mediates analgesia. Here we demonstrate that specific Toll-like receptors (TLRs) are up-regulated in response to CFA injection. This leads to macrophage infiltration into the dorsal root ganglia, and the production of interleukin 33 (IL33) which acts on sensory neuron to increase their activity. Block of spinal IL33/ST2 receptor signals attenuates CFA-induced inflammatory pain. The CFA induced upregulation of USP5 is abolished in TLR2 null mice, altogether indicating that the CFA mediated dysregulation of T-type calcium channel activity involves the activation of a TLR2-IL33-ST2 pathway, leading to the development of inflammatory pain.

The role of meningeal mast cells in ATP-induced nociceptive firing in trigeminal afferents. Anti-nociceptive effects of hydrogen sulfide

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ATP is one of the most prominent algogens, increasing the firing of trigeminal nerve, underling the migraine pain. Extracellular ATP released during migraine attacks activates meningeal afferents via neuronal P2X3 receptors. In addition ATP induces meningeal mast cell degranulation releasing serotonin which further induces nerve terminal excitation via 5-HT3 receptors. Thus, serotonin can be considered as the endogenous amplifier of purinergic nociception in meninges. Hydrogen sulfide (H2S) is a member of gasotransmitters family, induces both pro- and anti-nociceptive action in different tissues. It was shown that H2S donor -sodium hydrosulfide (NaHS) transiently increased firing in trigeminal nerve by activation of TRPV1 receptors however prevented pro-nociceptive effects of ATP. Moreover, H2S decreased currents and Ca2+ responses mediated by activation of P2X3 receptors in trigeminal neurons. Moreover, incubation of meninges in NaHS decreased ATP release and prevented mast cells degranulation. It was suggested than H2S may prevent pro-nociceptive effects of ATP in trigeminal system by inhibition of P2X3 receptors and stabilizing of meningeal mast cells. The work is supported by Russian Fund of Basic Research 18-315 -00256

2-S24-3 Symposium24

Brain-immune system and its maintenance of brain pain memory in fibromyalgia

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Although some medicines to suppress the pain symptoms in fibromyalgia have been recently developed and used in clinic, the treatments remain to be fully satisfied. One of reasons may be attributed to the long-term use, which may cause side effects. Based on this point of view, we are studying the systems pathology on chronic pain. We have proposed the hypothesis that feed-forward amplification of pain mechanisms plays key roles in neuropathic pain. One of evidence is that self-amplification of lysophosphatidic acid (LPA) production develops and maintains the pain memory in the peripheral neuropathic pain, which may be reinforced by neuro-inflammatory activation. On the analogy of the hypothesis in neuropathic pain, we have been studying the new mechanisms of central pain memory and its reinforcement system in fibromyalgia-like models. Fibromyalgia-like wide-spread centralized pain is developed by twice muscular injection with acidic saline, intermittent cold stress (autonomic) and intermittent psychological stress. All these wide-spread chronic pain diseases were completely cured by repeated brain injections of pregabalin or LPA receptor antagonist. In the present study we will report that peripheral immune system may reinforce the pain memory and it is also regulated by brain pain memory vice versa.

Neuroimmune crosstalk in neuropathic and visceral pain: HMGB1 and ATP as key mediators

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A neuroimmune crosstalk participates in diverse neuronal diseases including pathological pain. We have been studying the role of innate immunity involving DAMPs, such as HMGB1, a nuclear protein, and ATP in the development of chemotherapy-induced peripheral neuropathy (CIPN) and cystitis-related bladder pain. Intraplantar administration of HMGB1 causes long-lasting allodynia in rodents. Macrophage (Mϕ)-derived HMGB1 is involved in the development of CIPN following paclitaxel treatment. Paclitaxel directly causes HMGB1 release from Mϕ, which is promoted by ATP released from neurons stimulated with paclitaxel. Similarly, Mϕ-derived HMGB1 is involved in bladder pain accompanying cyclophosphamide (CPA)-induced cystitis in mice. Acrolein, a hepatic metabolite of CPA, triggers release of ATP from the urothelial cells, which in turn causes HMGB1 release from Mϕ. The extracellular HMGB1 induces NK-κB-dependent upregulation of cystathionine-γ-lyase, an H_2S-generating enzyme, in the urothelium by activating RAGE, and the generated H_2S enhances the activity of $Ca_v3.2$ T-type calcium channels expressed in nociceptors, resulting in bladder pain. Together, a neuroimmune cross talk mediated by DAMPs including HMGB1 and ATP appears to play a critical role in the development of CIPN and cystitis-related bladder pain.

Abnormal cell differentiation of human microglial cells and neuropsychiatric disorders: Translational research using human induced microglia-like (iMG) cells

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Postmortem brain analysis and PET imaging analysis are two major methods to estimate microglial activation in human subjects, and these studies have suggested activation of human microglia in the brain of patients with various psychiatric disorders. However, by using the above methods, only a limited aspect of microglial activation can be measured. We have originally developed a technique to create directly induced microglia-like (iMG) cells from fresh human peripheral blood monocytes adding GM-CSF and IL-34 for 2 weeks, instead of brain biopsy and iPS technique (Ohgidani, Kato et al. Sci Rep 2014). Using the iMG cells, dynamic morphological and molecular-level analyses such as phagocytosis and cytokine releases after cellular-level stress exposures are applicable. We believe that abnormal cell differentiation could be revealed using patient-derived iMG cells.

We have already revealed previously-unknown dynamic pathophysiology of microglia in patients with Nasu-Hakola disease (Sci Rep 2014), fibromyalgia (Sci Rep 2017) and rapid-cycling bipolar disorder (Front Immunology 2017). The iMG cells can analyze both state- and trait- related microglial characteristics of human subjects by repeated blood collection, which is especially valuable because majority of psychiatric disorders express situation- and time- oriented symptoms.

We believe that the iMG techniques shed new light on clarifying dynamic molecular pathologies of microglia in a variety of neuropsychiatric disorders.

Dedifferentiation of Schwann cells by taxanes participates in the chemotherapy-induced peripheral neuropathy pathogenesis

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Chemotherapy-induced peripheral neuropathy (CIPN) is a frequent side effect of taxanes. CIPN is a serious impediment in effective cancer treatment, because aggravation of symptoms results in discontinuation of taxane treatment or dose reduction, which can increase cancer related mortality and morbidity. Although impairment of neuroaxis of peripheral neurons by taxanes has been considered to be a major cause of CIPN, however, the precise underlying mechanisms are unknown. To address this issue, we have focused on the role of Schwann cells in supporting the maintenance of the peripheral nervous system, and have examined the direct effects of taxanes on these cells. We demonstrated for the first time that taxanes preferentially impair Schwann cells, rather than induce neurotoxicity in sensory neurons. Furthermore, the novelty of our study lies in the finding that paclitaxel induces dedifferentiation of myelin-forming Schwann cells characterized by increased expression of low affinity nerve growth factor receptor p75 (immature Schwann cell marker) and decreased expression of myelin-associated molecule (MBP, mature Schwann cell marker).
In this presentation, I will talk about our research on the mechanisms underlying CIPN pathogenesis, focusing on Schwann cells.

2-S25-3 Symposium25

Dynamic change in brain-microenvironment along with epigenetic modulation by exposure to stress

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Coordinated regulation of lipid metabolism and inflammatory response in macrophages

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Growing evidence has suggested that chronic inflammation is important for the pathogenesis of numerous diseases, including metabolic disorders and atherosclerosis. Macrophages play pivotal roles in chronic inflammation. We found that macrophages switch their cellular metabolism and functional phenotype throughout the course of inflammatory response. In response to inflammatory activation via Toll-like receptor (TLR4), macrophages rapidly activate glycolysis, increase inflammatory cytokine expression, acquire M1-like, pro-inflammatory phenotype. By contrast, macrophages increase unsaturated, anti-inflammatory fatty acid synthesis to show M2-like, anti-inflammatory phenotype at 24 hours following TLR4 activation. This late program of anti-inflammatory fatty acid biosynthesis is dependent on SREBP1 and results in the uncoupling of NF-kB binding from gene activation. Consistent with this, anti-inflammatory omega-3 fatty acids are decreased in *SREBP1*$^{-/-}$ macrophages, and systemic inflammation was prolonged in *SREBP1*$^{-/-}$ mice. These findings suggest the functional switch from M1-like to M2-like, and the metabolic switch from glycolysis to lipid metabolism are tightly linked and coordinately regulated during inflammatory response, and these temporal regulatory programs are important for proper inflammatory activation and resolution. Collectively, macrophages have endogenous, temporal programs to switch their function by linking inflammatory signals, and cellular metabolism. This program would be novel therapeutic target for atherosclerosis and metabolic syndrome.

Immuneregulation and tissue repair by Ym1^{+} monocytes

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急性炎症は、病原性異物の排除に必須の防御反応である。しかし、行き過ぎた炎症は２次的な組織傷害の原因となるため、炎症の原因除去後は遷延することのないよう、厳密に制御されている。組織に常在するマクロファージは、周囲環境に応答して柔軟にその形質を変化させることで、炎症の促進から収束への転換に寄与する。組織の常在構成員に加え、炎症の回復期には、組織外から派遣される免疫調節細胞の存在も想定されていたが、そのような細胞の実体は分かっていなかった。

我々は、定常状態や、炎症急性期にはほとんど存在しないが、炎症後期に骨髄で急激に増加し、炎症局所に移動するYm1陽性単球を発見した。この単球は、炎症調節型のサイトカイン産生パターンを示し、炎症で傷ついた組織の修復を促進することが分かった。この知見は、組織傷害時には、末梢組織から骨髄に向けて何らかのメッセージが発信され、それに応答して産生されるYm1陽性単球が、組織に動員されて恒常性維持に寄与することを示唆する。本講演では、我々によって新たに同定されたYm1陽性単球の機能と、炎症における役割について解説する。

2-S26-3 Symposium26

Cross-talking between EMT and inflammatory responses

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Epithelial-to-mesenchymal transition (EMT) is a process that converts adherent epithelial cells into motile mesenchymal-like cells and is known to be involved in metastasis/invasion and chemoresistance in cancer cells. Therefore, understanding of molecular mechanisms underlying EMT is beneficial for development of cancer therapies. Recently, we identified Vestigial-like family member 3 (VGLL3) as a novel EMT inducing protein by analyzing gene expression databases of the cells undergoing EMT with the stimulation of the cytokine TGF-b. VGLL3 is a transcriptional co-factor and binds to the transcription factor family TEAD. VGLL3 was shown to be induced by TGF-b stimulation and promote EMT through expression of the stem-cell factor HMGA2.

To understand molecular roles of VGLL3, we performed gene set enrichment analysis in VGLL3-expressing cells and found that VGLL3 activates inflammatory responses together with EMT signaling. Interleukin-1a expression was increased and NF-kB, a well-known pro-inflammatory signaling, was activated in VGLL3-expressing cells. We found that Interleukin-1a-NF-kB signaling promotes starvation-independent autophagy and that this autophagy is involved in cellular metabolic rewiring. These results suggest that the EMT-inducing factor VGLL3 activates starvation-independent autophagy and metabolic rewiring via inflammatory responses. We are currently analyzing involvement of these roles of VGLL3 in cancer progression.

Systemic environment regulates central nervous system regeneration

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Central nervous system (CNS) inflammation causes severer neurological dysfunction due to the damage of neuronal network. Regeneration of neuronal network is important to recovery from neurological dysfunction, but the mechanism of neuronal regeneration is not fully elucidated. Remyelination is the essential process of regeneration of neuronal network in the CNS, and we recently reported that remyelination is regulated by the circulating factors which leaks into the CNS after injury. In this talk, I will introduce our recent research that systemic environment promotes oligodendrocyte maturation, which is the last process of remyelination. We found that circulating Transforming growth factor (TGF)-beta1, which is present in higher levels compared with that in the CNS, stimulates oligodendrocyte maturation. TGF-beta mainly expresses in spleen and platelet. In the toxin-induced demyelination model, we revealed that treatment with neutralizing antibodies against TGF-beta prevents spontaneous remyelination. Also, platelet depletion experiments shows the inhibition of spontaneous remyelination in same demyelination model. We found that TGF-beta treatment promotes expression of myelin-associated proteins in human oligodendrocyte culture. These data suggest the possibility that circulating TGF-beta is beneficial for treating demyelinating diseases.