2-AS1-1

The outline and future outlook of MID-NET (Medical Information Database Network) ～ Toward the utilization of Real World Data ～

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MID-NET® is a medical information database that had been established under the governmental project of the Ministry of Health, Labour and Welfare since FY 2011, for a prompt and proper implementation of drug safety measures. PMDA has worked for developing database system, operating data quality management and establishing utilization rules. As a result, MID-NET® was officially launched in April 2018 and was opened for utilization by the pharmaceutical industry and academia.

MID-NET® is expected to become a major data source for more scientific and efficient post-marketing studies on drug safety assessment. We hope that MID-NET® plays an important role for promoting real world data utilization.

At this symposium, I will introduce the outline of MID-NET® including date quality management, utilization rules and future outlook of MID-NET®.

2-AS1-2
Evidence-based drug repositioning and target discovery

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FAERS is a public database that accumulates more than 9 million self-reports of adverse events. In nearly half of the cases, multiple drugs are prescribed, so that potential drug-drug interactions are to be analyzed. Focusing on adverse reactions relating to diabetes mellitus (DM) caused by an anti-schizophrenic quetiapine, we found that concomitant use of vitamin D analogs significantly suppresses the occurrence of quetiapine-induced DM in FAERS. Experimental validation revealed that quetiapine acutely caused insulin resistance, which was mitigated by dietary supplementation with cholecalciferol. In an expression database, several genes downstream of insulin receptor were downregulated by quetiapine. Further experiments clarified that a PI3K regulatory protein gene, pik3r1, was downregulated by quetiapine, which was reversed by cholecalciferol in mouse skeletal muscle. In addition, insulin-stimulated glucose uptake into cultured myotubes was inhibited by quetiapine, which was reversed by pretreatment with calcitriol. These results suggest that vitamin D prevents the atypical antipsychotic-induced hyperglycemia and insulin resistance by upregulation of PI3KR1. Until now, we have obtained several combinations of concomitant medications to reduce specific adverse events by FAERS analysis. This new strategy will pave the way for drug repositioning and clarifying unknown disease mechanisms.

2-AS1-3

Search for preventive drugs against anticancer drug-induced side effects using a large-scale medical information database

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Treatment outcomes of cancer patients have improved with progress in oncology medication therapy, but side effects caused by anticancer agents are becoming widespread. Side effects caused by anticancer drugs not only significantly lower the patient's QOL but also often lead to dose reduction or discontinuation of the anticancer drugs. Addressing these side effects is important for improving patient prognosis. Therefore, improvement of the quality of cancer therapy through the development of preventive drugs against anticancer drug-induced side effects is an urgent goal. In recent years, clinical research has been carried out in Japan using large-scale medical information sources such as disease/side effect databases, in order to accurately evaluate the effects of drug used in clinical practice. Research utilizing such a large-scale medical information database can cover various patient parameters and a wide range of observation areas. Therefore, this approach is suitable for conducting clinical research on rare diseases and low-frequency side effects. In this symposium, we will introduce research conducted using drug discovery tools and cell/animal experiments based on a large-scale medical information database to search for preventive agents against anticancer drug-induced side effects, as well as consider future prospects for this approach.

2-AS1-4
The use of Real-World Data in drug development

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The Health, Care and Educational Information Evaluation Promotion Organization (HCEI) has established a database centered on electronic medical records in collaboration with 160 medical institutions since 2015. As a characteristic of this database, it contains not only data of DPC and receipt already, but also test results and death data. As a result, we can conduct outcome research to measure the effects of drugs and treatments.
Our database is not dependent on vendors and register in the database about 19 million patients. Based on JLAC10, it has standardized 1000 kinds of inspections (with unit / sample classification), and it becomes a useful database for post marketing surveillance etc.
In addition, we have also started a project to collect data in the registry and the data extraction in the randomized controlled trial conducted by the academic society, and we are promoting efforts to eliminate the load of data extraction in the hospitals. Since effective utilization of medical data is a major cornerstone for drug discovery or quality improvement of medical treatment, we would like to continue to develop the database for the promotion of primary and secondary use of medical information.

2-AS2-1
Integrative analysis of public omics databases for drug discovery

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The rapid advances in high-throughput technologies have facilitated the collection of multilevel omics data. The increasing volume of multilevel omics data continues to create larger and more complex datasets which are publicly available and can be used to generate disease-associated biological networks and to identify potential therapeutic targets within the networks. Further progress in computational methodology combined with improved disease models will facilitate the prioritization of therapeutic targets in the networks. The integration of public omics database, bioinformatics tools, and disease models can provide a strong foundation for deciphering the complex mechanism of various diseases and for data-driven drug discovery. In this symposium, I would like to demonstrate some examples of how the integrative approaches using public omics databases and animal disease models can be exploited to identify potential therapeutic targets for various disorders.

2-AS2-2
Data-driven and pathway-based drug discovery by machine learning

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Drug repositioning, or the identification of new indications of drugs (i.e., new applicable diseases), is an efficient strategy for drug development, and it has received remarkable attention in pharmaceutical science. The drug repositioning approach can increase the success rate of drug development and to reduce the cost in terms of time, risk, and expenditure. In this study, we developed novel machine learning methods for automatic drug repositioning in order to predict unknown therapeutic indications of known drugs or drug candidate compounds. We also proposed to use molecular pathways as the therapeutic targets and develop novel computational approach for screening drug candidate compounds. The prediction is performed based on the analysis of various large-scale omics data and molecular interaction networks of drugs, compounds, genes, proteins, and diseases in a framework of supervised network inference. Our results show that the proposed method outperforms previous methods in terms of accuracy and applicability. We performed a comprehensive prediction of new indications of all approved drugs and bioactive compounds for a wide range of diseases defined in the International Classification of Diseases. We show several biologically meaningful examples of newly predicted drug indications for cancers and neurodegenerative diseases. The proposed methods are expected to be useful for various pharmaceutical applications in drug discovery.

2-AS2-3

Platform type of drug repositioning/rescue by mimicking cell-state change

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We developed a platform for drug repositioning/rescue by using omics data that express the cell-state changes by drug administration, based on the concept of well-known "connectivity map", which the transcriptome data by drug administration is connected to drug efficacy. In addition to connectivity-map approach, the platform is equipped with an original method to estimate the drug efficacy based on the network analyses. Furthermore, the platform is also equipped with a workflow to estimate new stratification markers of the indications newly found by drug repositioning. Note that in principle, the platform type of drug repositioning/rescue can provide solutions of commercialized drugs against any kind of target diseases. We will illustrate our platform performance with some practical applications, especially in terms of the increase of certainty of new findings, at the spot.

Symposium

1-S01-1
Overview of current status of Central Neuro-Uro-Pharmacology

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The functions of the lower urinary tract (LUT), to store and periodically release urine, are dependent on the activity of smooth and striated muscles in the urinary bladder, urethra, and external urethral sphincter. This activity is in turn coordinated by neural circuits in the central nervous system (CNS). Various neurotransmitters, including acetylcholine, norepinephrine, dopamine, serotonin, excitatory and inhibitory amino acids have been implicated in the CNS regulation of the LUT. Injuries or diseases of the CNS such as cerebral infarction, Parkinson disease, multiple sclerosis and spinal cord injury, as well as psychological stress or depression, can produce LUT dysfunctions leading to storage and voiding LUT symptoms such as urinary frequency, urgency, pain and incontinence or inefficient voiding and urinary retention. The recent research advancement in the field of CNS neuro-urology has led to the emergence of new concepts regarding neural control of the LUT and the etiology of LUT dysfunction. In this symposium, I will overview the recent advancement towards the identification of disease-related changes in receptor function and new delivery systems such as gene therapy techniques, which could lead to the future treatment of LUT dysfunction.

Symposium

1-S01-2

Effects of phosphodiesterase type 5 inhibitor and alpha-1A/D adrenoceptor antagonist on bladder remodeling in rats with spinal cord injury

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Spinal cord injury (SCI) can lead to detrusor overactivity and detrusor-sphincter dyssynergia, which result in inefficient voiding and bladder wall tissue remodeling such as hypertrophy and fibrosis. However, no effective modality for controlling the bladder remodeling is available. Phosphodiesterase type 5 (PDE5) inhibitors and alpha1-adrenoceptor (α_1-AR) antagonists are used for the treatment of male lower urinary tract symptoms with benign prostatic hyperplasia.

In animal study, PDE5 inhibitor or $\alpha_{1A/D}$-AR antagonist treatment suppressed the bladder fibrosis after SCI. PDE5 inhibitor might increases the blood flow and prevents bladder ischemia, resulting in the reducing the load in storage state and the suppressing of bladder fibrosis after SCI. Relaxing the bladder neck and proximal urethra after α_1-AR antagonist treatment might decrease the resistance to urine flow from detrusor–sphincter dyssynergia and residual urine volume reduce the load of the bladder wall during both of voiding and storage states. Therefore, treatment with PDE5 inhibitors and $\alpha_{1A/D}$-AR antagonists could be effective for neurogenic lower urinary tract dysfunction including bladder remodeling after SCI.

Symposium

1-S01-3

Therapeutic targets for stress urinary incontinence in the central nervous system

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Stress urinary incontinence (SUI) is a common and bothersome problem among middle-aged women. Various animal models of SUI have been developed to study the pathophysiological process involved in SUI, such as vaginal distention, pudendal nerve injury, or ovariectomy. We have also reported cerebral infarction rats induce not only bladder overactivity, but also SUI. Leak point pressure measurements are the most commonly used methods to evaluate the urethral dysfunction in SUI animal models. Originally, we have developed microtransducer-tipped catheter measurements of urethral activity during sneezing. Extensive our basic research has clarified potential strategies for pharmacotherapy of SUI in the central nervous system. Therapeutic targets include adrenergic and serotonergic (5-HT) receptors in the spinal cord projected from neurons in the locus coeruleus and raphe nucleus, respectively, which stimulate pudendal nerve innervating the external urethral sphincter. Activation of α_1-adrenoceptors, 5-HT_{2C}, or 5-HT_7 receptors enhances the reflex at the spinal cord level whereas pre- or postsynaptic α_2-adrenoceptors and/or 5-HT_{1A} receptors inhibit the reflex. In addition, we have reported that stimulation of the spinal μ-opioid receptors may be a new candidate for the treatment of SUI. Thus, we review the recent advances in basic SUI research and potential targets for pharmacotherapy of SUI in the central nervous system.

Symposium

1-S01-4

Central regulation mechanisms for stress-induced frequent urination

Takahiro Shimizu[1], Shogo Shimizu[1], Youichirou Higashi[1], Naoki Yoshimura[2], Motoaki Saito[1]

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Psychological stress exacerbates symptoms of bladder dysfunction including overactive bladder and bladder pain syndrome/interstitial cystitis not only in rodent models but also in human patients. Bombesin (BB)-related peptides and BB receptors in the brain have been implicated in the mediation/integration of stress responses. We have found that brain BB induces frequent urination through the BB receptors, serotoninergic nervous system and corticotropin-releasing factor (CRF) receptors. Interestingly, the BB-induced response is independent of the BB-induced activation of the sympatho-adrenomedullary outflow, one of the components of the primary systems for maintaining or reinstating homeostasis during stress exposure. These findings indicate that brain BB, 5-HT and CRF receptors could be new therapeutic targets for bladder dysfunction exacerbated by stress exposure. There are several concepts regarding central regulation mechanisms for the bladder function in the normal and pathological conditions. However, the exact brain pathophysiological mechanisms underlying stress-induced effects on the bladder are largely unknown. Therefore, our findings could pioneer a novel neuropharmacological field, central "Neuro-Uro-Pharmacology".

Symposium

1-S02-1
Significance of iron and therapeutic application in diseases

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Iron is an essential trace metal element, and it is involved in hemoglobin synthesis, redox reaction, enzyme activity, cell proliferation and apoptosis in various cells. Therefore, iron overload has not attracted attention as compared with iron deficiency typified by anemia. Excessive iron produces hydroxyl radicals with strong oxidizing power via Fenton reaction, causing organ damage in hereditary iron overload diseases. Recent years, it has been clarified that iron accumulation is involved in the pathological conditions even in diseases including cardio-renal vascular diseases and metabolic diseases thought to be unrelated to iron so far. Therefore, the role of iron in the living body has been raised attention again.

We have revealed that iron reduction by iron chelator ameliorates adipose oxidative stress, contributing to the reduction of fat hypertrophy, macrophage infiltration and inflammatory cytokine expression in obese and diabetic KKAy mice. Dietary iron restriction also diminishes renal oxidative stress, leading to the inhibition of albuminuria excretion, glomerular lesions and inflammatory cytokines in *db/db* mice, a model of diabetic kidney disease.

In the presentation, we'd like to outline the role of iron on obesity diabetes and its complication, and the possibility of application to treatment with iron regulation in those disorders.

Symposium

1-S02-2

Development and applications of fluorescent probes for the detection of Fe(II) ion

Tasuku Hirayama[1]

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Iron is the most abundant transition metal in our body and plays various pivotal roles in our lives including oxygen transport, energy production, and metabolic reactions. At the same time, an excess amount of iron may cause cellular damages through undesired oxidative reactions. We have developed several fluorescent probes, which can detect Fe(II) ion selectively with fluorescence enhancement, to understand both the physiological and pathological contributions of iron in living systems. These fluorescent probes worked in an aqueous buffer, living cells, and histochemical samples (*Chem. Sci*. 2013, 4, 1250; *Chem. Sc*i. 2017, 8, 4858; *Free Radic. Res.* 2014, 48, 990; *Sci. Rep*. 2017, 7, 10621) I will present the recent progress of the development of organelle-targeted fluorescent probes for mitochondria, lysosome, and plasma-membrane (*Metallomics*, 2018, 10, 794; *Metallomics*, 2018, *in press*; *ACS Chem. Biol.*, 2018, 13, 1853). Besides, our recent approach to discover iron-regulating drug by high-throughput screening with a highly sensitive fluorescent probe will be presented.

Symposium

1-S02-3
Physiological and pathophysiological relevance of zinc transporters

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Zinc is an essential trace element that is required for a variety of cellular functions, and unbalanced zinc homeostasis results in health problems. Recent studies have highlighted that zinc acts as a signaling mediator: zinc signal, which is controlled via zinc transporters, and participates in health and disease conditions **(1)**. In this symposium, I will address the updated information about the roles of zinc homeostasis and zinc signaling in physiology and pathophysiology.

The first manifestations that appear under zinc deficiency are skin defects **(2)**. It should be also noted that about 60% of whole zinc in body is kept in skeletal muscle. In addition, attentions have been drawn to zinc accumulation in tumors. However, the physiological and pathophysiological relevance, and their zinc-related molecular mechanisms underlying normal skin and muscle development, as well as the mechanism by which disturbed zinc homeostasis causes disorders including cancers, have not been clarified yet.

In this symposium, I will provide an overview of the relationships between zinc dysregulation and skin disorders, by focusing on the roles of zinc transporter ZIP7 and ZIP10 in skin formation **(3, 4)**, and cancer cachexia mediated by ZIP14 **(5)**. I also address the zinc homeostatic system contributes skeletal muscle formation and function via zinc signaling mediated by ZIP13.

References:

1: ***International Journal of Medical Sciences*** 18: 2708, 2017
2: ***Nutrients*** 10: 219, 2018
3: ***Journal of Investigative Dermatology*** 137: 1682-1691, 2017
4: ***Proc. Natl. Acad. Sci. USA*** 114:12243-12248, 2017
5: ***Nature Medicine*** 24:770-781, 2018

Symposium

1-S02-4

Elementomics reveals the accumulation of copper in the brain of a Down syndrome model mouse and its pathophysiological significance

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Down syndrome (DS) with an additional copy of the human chr.21 (HSA21) is characterized by various phenotypes, such as intellectual disability and neurological abnormalities. Although it is widely accepted that a high level of oxidative stress (OS) is involved in DS symptoms, the actual role of elevated OS on DS phenotypes remains unclear. Among the genes on HSA21, amyloid-β precursor protein (App) and Cu/Zn superoxide dismutase (Sod1) are thought to be involved in the elevated OS and neurological abnormalities commonly described in DS. Ts1Cje mice, a widely used genetic model of DS, exhibit some of the abnormalities of DS patients, including cognitive impairment with electrophysiological abnormality, despite the exclusion of App and Sod1 in their trisomic region. We demonstrated a high level of OS in Ts1Cje mice, suggesting that the trisomic gene(s) other than App and Sod1 increase the OS in Ts1Cje mice. In this symposium, I intend to introduce our latest results, which suggest the accumulation of copper in the Ts1Cje mouse brain, and discuss the pathophysiological significance of copper in relation to the abnormalities observed in DS patients, particularly elevated OS.

Symposium

1-S03-1

Involvement of transient receptor potential vanilloid 4 (TRPV4) in the regulation of murine colitis and colitis-associated cancer

Kenjiro Matsumoto[1], Shinichi Kato[1]

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TRPV4 is a nonselective cation channel and involved in physical sensing in various types of tissues. The present study investigated the roles of TRPV4 in the pathogenesis of colitis and colitis-associated cancer in mice. The severity of colitis induced by daily treatment with dextran sulfate sodium (DSS) was significantly attenuated in TRPV4-deficient mice when compared with wild-type (WT) mice. The up-regulation of endothelial TRPV4 in colitis models suggests that this channel plays a crucial role in intestinal inflammation via increasing vascular permeability. TRPV4 activation decreased the major endothelial adhesion molecule VE-cadherin in the mouse colon and mouse aortic endothelial cells. The formation of colonic cancer induced by azoxymethane injection following DSS-treatment was also significantly attenuated in TRPV4-deficient mice. TRPV4 was co-localized with f angiogenesis marker and macrophages marker. Azoxymethane/DSS-treatment upregulated the expression of CD105 and VEGFR2 as well as TRPV4 in WT, but these responses were significantly attenuated in TRPV4-deficient mice. Activation of TRPV4 increased TNF-α and CXCL2 expression in peritoneal macrophages. These results suggest that TRPV4 expressed in vascular endothelia and macrophages contribute to progression of colitis and colitis associated cancer.

Symposium

1-S03-2

Role of low voltage-activated $Ca_v3.2$ T-type Ca^{2+} channels in prostate cancer cells

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$Ca_v3.2$ T-type Ca^{2+} channels regulate neuronal excitability, contributing to pain signaling, and their function is enhanced by H_2S, a gasotransmitter. $Ca_v3.2$ is also expressed in certain cancer cells, and may affect cancer patients' prognosis. We have been studying the role of $Ca_v3.2$ in human prostate cancer LNCaP cells, particularly during neuroendocrine (NE)-like differentiation, which might be involved in hormone therapy resistance of prostate cancer. $Ca_v3.2$ and cystathionine γ-lyase (CSE), an H_2S-generating enzyme, are overexpressed in LNCaP cells during NE differentiation, accompanied by upregulation of Egr-1 and downregulation of REST, which contribute to transcriptional upregulation of $Ca_v3.2$. These events may participate in increased secretion of mitogenic factors essential for proliferation of surrounding cells. Further, increased extracellular glucose levels accelerate functional upregulation and overexpression of $Ca_v3.2$ probably through asparagine-linked glycosylation of $Ca_v3.2$. Our study thus suggests a crucial role of the $H_2S/Ca_v3.2$ system in NE-like differentiated LNCaP cells, which is promoted by hyperglycemia, being consistent with the clinical evidence that diabetes is a risk factor for castration-resistance of prostate cancer.

Symposium

1-S03-3

Cancer cell-specific functional relation between Na,K-ATPase and volume-regulated anion channel

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Cardiac glycosides such as digitoxin and digoxin are Na,K-ATPase inhibitors, and they have been used clinically for treatment of heart failure. Interestingly, lower recurrence of cancer and improvement of their survival are suggested in cancer patients who have been taking cardiac glycosides. In the cancer cells, it has been reported that sub-micromolar cardiac glycosides showed anti-cancer effects without affecting Na,,K-ATPase activity. We found recently that the receptor-type Na,K-ATPase, which has no pumping activity, is specifically associated with LRRC8A, an component of volume-regulated anion channel (VRAC), in the membrane microdomains of plasma membrane of cancer cells, and that functional relation between them is involved in the inhibitory mechanism of submicromolar cardiac glycosides for cancer cell growth. In this mechanism, binding of cardiac glycosides to the receptor-type Na,K-ATPase stimulates the production of reactive oxygen species by NADPH oxidase, and they activate VRAC within membrane microdomains, thus eliciting anti-proliferative effects. Of note, digoxin and digitoxin showed anti-proliferative effects in cancer cells at their therapeutic concentration ranges. These effects were not observed in non-cancer cells.

Symposium

1-S03-4

Ca^{2+}-activated K^+ channel as potential therapeutic target for cancer treatment

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In cancer cells, Ca^{2+}-activated K^+ channels $K_{Ca}1.1$ and $K_{Ca}3.1$ are co-localized with Ca^{2+}-permeable Orai/TRP channels to provide a positive-feedback loop for Ca^{2+} entry. They are responsible for the promotion of cell growth and metastasis in the different types of cancer, and are therefore potential therapeutic targets and biomarkers for cancer. We determined the epigenetic and post-transcriptional dysregulation of $K_{Ca}3.1$ by class I histone deacetylase (HDAC) inhibitors in breast and prostate cancer cells. We further determined the transcriptional repression and protein degradation of $K_{Ca}1.1$ by vitamin D receptor (VDR) agonists and androgen receptor (AR) antagonists, which are expected as potential therapeutic drugs for triple-negative breast cancer. A class III HDAC, SIRT1 is involved in cancer growth, stemness, and metastasis. We here introduce SIRT1-mediated regulation of $K_{Ca}3.1$ expression in breast, prostate, and colorectal cancer by three-dimensional spheroid culture. The anti-inflammatory cytokine, interleukin-10 (IL-10) is an immunosuppressive factor involved in tumorigenesis, and plays a crucial role in escape from tumor immune surveillance. We determined $K_{Ca}3.1$ activators are a possible therapeutic option to suppress the tumor-promoting activities of IL -10. These results may provide new insights into cancer treatment focused on Ca^{2+}-activated K^+ channels.

Symposium

1-S04-1

Arrhythmias caused by abnormal Ca^{2+} homeostasis and the treatment strategies

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Type 2 ryanodine receptor (RyR2) is the Ca^{2+} release channel on the ER and plays a pivotal role in EC-coupling in the heart. Abnormal activation of RyR2 has been implicated in arrhythmogenic diseases. For example, in heart failure, chronic phosphorylation of RyR2 can contribute to enhanced Ca^{2+} leak from ER. In addition, mutations in RyR2 are reported to cause arrhythmogenic diseases such as catecholaminergic polymorphic ventricular tachycardia (CPVT) and long QT syndrome (LQTS). In most cases spontaneous Ca^{2+} release from ER via activated RyR2 is thought to trigger arrhythmia. We have recently established a procedure for functional evaluation of the arrhythmogenic RyR2 mutations using HEK293 expression system. Furthermore, we identified several compounds that suppress RyR2 activity via high-throughput screening using the HEK293 system. Because some of them suppressed abnormal Ca^{2+} signals in mouse cardiomyocytes, RyR2 inhibitors may be promising as novel anti-arrhythmic drugs.

Symposium

1-S04-2

Ca^{2+} signaling and ion channels in pulmonary arterial hypertension

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Pulmonary arterial hypertension (PAH) is classified as group 1 of pulmonary hypertension. PAH is a progressive and fatal disease of the pulmonary artery. The major pathogenesis of PAH is sustained vasoconstriction and vascular remodeling of the pulmonary artery. These pathogeneses cause progressive elevations in pulmonary vascular resistance and pulmonary arterial pressure (PAP) in PAH patients. Elevated PAP leads to right heart failure and finally death. A central aspect of pulmonary vascular remodeling is medial hypertrophy, which is caused by the enhanced proliferation and reduced apoptosis of pulmonary arterial smooth muscle cells (PASMCs). Excitable abnormality in the pulmonary artery of PAH patients are mostly mediated by an elevated cytosolic [Ca^{2+}]. Enhanced Ca^{2+} signaling have been reported in PASMCs from PAH patients. PASMCs express several Ca^{2+}-permeable channels including voltage-dependent Ca^{2+} channels, receptor-operated Ca^{2+} channels, and store-operated Ca^{2+} channels. The expression levels of these Ca^{2+} channels are increased in the lung tissues and PASMCs of PAH patients. Targeting these Ca^{2+} channels in PASMCs may help develop novel therapeutic approach for PAH.

Symposium

1-S04-3

Role of functional foods material on skeletal muscle homeostasis

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The polyamines, a functional foods material are considered to be essential growth factors in virtually all cells. The proposed roles of polyamines are the functioning of ion channels, nucleic acid packaging, signal transduction, cell proliferation, and differentiation, as well as regulation of gene expression. In skeletal muscle, regulation of polyamine levels is associated with muscle hypertrophy and atrophy, yet the underlying mechanisms of polyamine actions are not well defined. Here, we studied how polyamines may affect the proliferation and/or differentiation of murine myoblast progenitor C2C12 cell line. Upon polyamine treatment of C2C12 cells during induction of myogenic differentiation, the number of myotubes significantly increased. Morphologically, polyamine-treated C2C12 cells exhibited elongated cell body and became multi-nucleated myotubes. On the other hand, the polyamine did not have influence on myoblasts proliferation. Furthermore, compensatory muscle hypertrophy of C57BL6 mice underwent sciatic nerve transection of the left hindlimb was enhanced by administration of polyamines. Therefore, our study demonstrates that polyamines may play an important role in skeletal muscle homeostasis by enhancing myogenic differentiation.

Symposium

1-S04-4

Signaling mechanism of myoblast fusion in skeletal muscle formation

Taichiro Tomida[1], Kimitaka Yamaguchi[1], Masanori Ito[1], Yoshinori Mikami[1], Daisuke Ohshima[1], Satomi Adachi-Akahane[1]

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Formation of skeletal muscle occurs at the stage of embryonic development as well as growth and regeneration in the adult. A skeletal muscle cell forms an elongated fiber containing multiple nuclei that are originated by fusion of precursor myoblasts. Recently, skeletal muscle-specific fusogenic transmembrane proteins Minion and Myomaker were identified and the mice lacking these factors showed severe muscle loss phenotype. In this study, we aimed to investigate the regulatory mechanism of the fusogenic proteins. C2C12 myoblast cell line was used as a model for *in vitro* myogenesis. The cells became elongated then fused to form multinucleated myotubes within 72 hours after induction of differentiation. By challenging kinase inhibitors previously known to affect muscle development, we found that activity of p38 MAPK but not ERK, JNK, ERK5 is required for induction of both Minion and Myomaker. Actually, the activity of p38 MAPK was continuously increased during C2C12 differentiation, and application of p38 inhibitor abrogated cell-cell fusion. Knockout of *minion* gene in C2C12 abrogated efficient fusion, but these cells were capable of inducing Tn-T, suggesting that myoblast fusion can be regulated independently of its differentiation. As p38 MAPK is a key mediator of stress and inflammatory signaling, it is possible that p38 mediated fusion process contributes to muscle regeneration after injury or might be involved in the pathology of abnormal muscle loss with increased inflammatory cytokines in cachexia.

Symposium

1-S05-1

Built in "osteo-innate-immunological machinery" in nociceptive system

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Candida albicans infection can cause skin, vulvar, or oral pain. Despite the obvious algesic activity of C. albicans, the molecular mechanisms of fungal nociception remain largely unknown. Here we show that the C. albicans-specific signaling pathway led to severe mechanical allodynia. We discovered that C. albicans-derived β-glucan stimulated nociceptors depending on Dectin-1, and two pathways in inflammatory pain. The major pathway operates via the Dectin-1-mediated ATP-$P2X_3$/$P2X_{2/3}$ axis through intercellular relationships between keratinocytes and primary sensory neurons, which depends on the ATP transporter vesicular nucleotide transporter (VNUT). The other pathway operates via the Dectin-1-mediated PLC-TRPV1/TRPA1 axis in primary sensory neurons. Intriguingly, C. albicans-derived β-glucan has the ability to enhance histamine-independent pruritus, and VNUT inhibitor clodronate can be used to treat unpleasant feelings induced by β-glucan. Collectively, this is the first report to indicate that Dectin-1 and VNUT mediated innate sensory mechanisms that detect fungal infection.

Symposium

1-S05-2

Modification of tumor aggravation via the tumor microenvironment by the reflection/activation of sensory nerves

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A growing body of evidence suggests that intractable pain is associated with a decreased survival rate with a reduced quality of life in cancer patients. Recently, early palliative care has been shown to dramatically improve the survival rate of cancer patients. The tumor microenvironment refers to the cellular environment in which the tumor exists, including surrounding blood vessels, immune cells, fibroblasts and sensory neurons. Among those, the vascular and nervous systems share some critical guidance molecules. These comprehensive functions may ultimately affect tumor aggravation. It is of interest to note that the release of neurotransmitters including many of growth factors from sensory nerves could lead to neurogenic inflammation and may result in tumor progression. Therefore, we hypothesize that the activation of sensory nerves may contribute to tumor aggravation via the tumor microenvironment. In this session, we will introduce recent critical findings that could prove this hypothesis and discuss the possible mechanism of tumor growth associated with pain.

Symposium

1-S05-3

Control of somatosensory behavior by a subpopulation of spinal dorsal horn astrocytes

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Astrocytes are critical regulators of CNS function. It has recently been proposed that astrocytes are a heterogeneous population in the developing CNS. However, whether there are regionally and functionally distinct populations of astrocytes in adulthood and if so, whether they play a role in behavior are unknown. Here we identify a population of astrocytes located in superficial lamina in the adult spinal dorsal horn (supSDH). In vivo imaging revealed that supSDH astrocytes increased intracellular Ca^{2+} levels following noxious stimulation such as by capsaicin. To examine the role of astrocytic Ca^{2+} increases in somatosensory behavior, we chemogenetically increased Ca^{2+} in supSDH astrocytes produce pain hypersensitivity to light mechanical stimulation. However, such astrogliogenic hypersensitivity was not induced by stimulating astrocytes located in SDH deeper lamina. Moreover, mechanical hypersensitivity following intraplantar capsaicin was prevented by lacking IP_3R2, an IP_3R subtype that critically contributes to astrocytic Ca^{2+} responses. Our findings identify a regionally restricted astrocyte population in the supSDH that powerfully modulates neuronal processing of mechanical information.

Symposium

1-S06-1

Exploring the applicability of regenerative medicine technology using human embryonic or induced pluripotent stem cells to the diagnosis and treatment of arrhythmia

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Diagnosis and treatment of arrhythmia are not sufficiently supported by present medical care. Therefore, we studied the applicability of human embryonic (ESC) or induced pluripotent stem cells (iPSC) to the diagnosis and treatment of arrhythmia. In the study on the diagnosis of arrhythmia, we established disease-specific iPSCs from a patient with long QT syndrome type 1 (LQTS1) and confirmed electrophysiological properties of LQTS1 after the cardiac differentiation. Computer simulation of the sympathetic activation state revealed the occurrence of early afterdepolarizations, the cause of sudden cardiac death in LQTS. In the study on the treatment of arrhythmia, we selectively obtained pacemaker-, Purkinje-, ventricular myocyte-like cells from cells differentiated from human ESCs/iPSCs using HCN4 and Mlc2v genes as labeling genes. When implanted to a rat model with atrioventricular block, the pacemaker-like cells successfully functioned as a pacemaker with an ability to respond to sympathetic stimulation. In conclusion, the application of human ESCs/iPSCs to the diagnosis of arrhythmia is promising and that to the treatment of arrhythmia is feasible.

Symposium

1-S06-2

The strategy of molecular therapy for neuronal diseases with the application of cell migration and targeting

Tomoya Terashima[1]

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In neuronal diseases, it is difficult to provide the satisfactory outcome only by stem cell transplantation because their pathogenesis is complex. Therefore, it should be developed a novel therapy considering the pathophysiology. We focused on migration of bone marrow-derived cell (BMDC)s to pathological lesion step by step with disease progression. We devised to apply this phenomenon, namely cell migration and targeting, to therapeutic strategy.

As application of cell migration, bone marrow transplantation was performed for the treatment of ALS mice. BMDCs migrated to the spinal cord and delayed disease progression, and this effect was enhanced by stem cell factor. Furthermore, we show other strategies with mesenchymal stem cells expressing growth factors transduced by human artificial chromosome vectors and with BMDCs as gene delivery carrier to target tissues.

As application of cell targeting, we have identified tissue specific peptides for dorsal root ganglion, microglia or astrocytes. And their peptides were incorporated into viral vectors or the complexes with therapeutic oligonucleotides to develop a novel therapy.

The combination of cell migration and targeting is a very useful tool for novel molecular therapy of neuronal diseases. This strategy is expected to have high therapeutic potential because therapeutic genes are targeted to specific cells and rescue cells gradually accumulate in pathological lesion as much as diseases progress.

Symposium

1-S06-3

Development of cell therapy for muscular dystrophy by iPSC-derived muscle stem cell

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Cell therapy is one of desired method for treating intractable muscular diseases, such as Duchenne muscular dystrophy (DMD). Here, we demonstrated the effective stepwise differentiation method from human iPSCs to engraftable muscle stem cells without transgene induction. We induced myotome-like population that is identified as Myf5 positive cells, which showed highly myogenic differentiation potential in vitro. Gene expression profile of purified Myf5+ cells demonstrated that the expression of Pax7 was significantly increased in Myf5+ cells at the late stage of differentiation. To assess the regeneration potential, we transplanted the Myf5+ cells at the late stage of differentiation into immunodeficient DMD-model mice. The Myf5+ cells could be engrafted in more than one hundred of host myofibers and regenerate the diseased muscles with producing dystrophin. Finally, we confirmed the recovery of muscle function after transplantation. Taken together, we demonstrate that the transplantation of the human iPSC-derived muscle stem cells with step-wise differentiation can be effective for DMD with amelioration of muscle function.

Symposium

1-S06-4

Novel therapeutic strategy for kidney and endocrine diseases using iPS cell technology

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The differentiation of induced pluripotent stem cells (iPSCs), which have unlimited self-renewal capability and the potential to differentiate into any cell type in the body, provides promising cell sources for regenerative medicine. The somatic cell types differentiated from these stem cells have the potential for clinical applications, including cell therapy and drug screening. In this symposium, I will briefly summarize our novel therapeutic strategy for kidney and endocrine diseases using iPS cell technology.

Firstly, I will present our recent findings about cell therapy for renal anemia. The production of erythropoietin (EPO), a principal hormone for the hematopoietic system, by the kidneys is reduced in patients with chronic kidney disease (CKD), eventually resulting in severe anemia. Although recombinant human EPO treatment improves anemia in patients with CKD, returning to full red blood cell production without fluctuations does not always occur. Although vigorous efforts have been made to generate multiple somatic cell types from stem cells, the directed differentiation of EPO-producing cells (EPO cells) from iPSCs has not yet been achieved. Recently, we established a method to generate EPO-producing cells from human iPSCs (hiPSCs) by modifying previously reported hepatic differentiation protocols. These cells showed increased EPO expression and secretion in response to low oxygen conditions. The EPO protein secreted from hiPSC-derived EPO-producing (hiPSC-EPO) cells induced the erythropoietic differentiation of human umbilical cord blood progenitor cells *in vitro*. Furthermore, transplantation of hiPSC-EPO cells into mice with CKD induced by adenine treatment improved renal anemia. Thus, hiPSC-EPO cells may be a useful tool for clarifying the mechanisms of EPO production and may be useful as a therapeutic strategy for treating renal anemia.

Secondly, I will mention about drug screening and evaluation for renal anemia. It has been reported that EPO production is regulated by oxygen concentrations through hypoxia-inducible factors and their regulators, prolyl hydroxylase domain-containing enzymes (PHDs). Several PHD inhibitors are currently in clinical trials for treatment of renal anemia. Interestingly, our recent findings showed that a PHD inhibitor augmented EPO production only in hiPSC-EPO cells, but not HepG2 cells, which are an immortalized human hepatoma cell line and are widely used to investigate EPO production. These findings suggest that hiPSC-EPO cells may provide a good model for screening PHD inhibitors for their effects on renal anemia.

Finally, I will brief summarize recent topics about cell therapy for endocrine disease using iPS cell technology.

Symposium

1-S07-1

The 3D imaging of cytoskeletons by IRIS, multi-target super-resolution microscopy with high density labeling

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The 3D networks of cytoskeletons are mechanistic bases for cell and tissue function (e.g. epidermal barrier function). To examine the details of the 3D networks, we extended multi-target super-resolution microscopy IRIS (Kiuchi et al., Nat. Methods 12: 743-746, 2015) to 3D imaging. IRIS uses exchangeable probes that directly associate with and dissociate from their targets. By integrating single-molecule localization and sequential labeling, IRIS enables the high resolution imaging of multiple targets within a single specimen. By the repeated associations of the probes, the loss of fluorescent signals due to photobleaching during the 3D imaging can be compensated. Moreover the labeling density on the target structures can be unlimitedly increased, yielding greatly improved continuous labeling along the cytoskeletal filaments. We developed IRIS probes for three cytoskeletons and focal adhesions from the target-associated proteins. For 3D IRIS imaging, we combined the IRIS method with HILO illumination and astigmatism using adaptive optics. This allowed us to discern microtubules at heights of 6.3 μm and 6.5 μm in the apical region of the cell. Thus IRIS is a potent tool to monitor the 3D network of cytoskeletons.

Symposium

1-S07-2
Destabilization of cell identities by transcription factor Srf

Takashi Ikeda[1], Takuya Yamamoto[1], Yasuhiro Yamada[1,2], Shinji Masui[1,3], Keisuke Okita[1]

[1]Center for iPS Cell Research and Application, Kyoto University, [2]Institute of Medical Science, The University of Tokyo, [3]Department of Ophthalmology, Kyoto Prefectural University of Medicine

Multicellular organisms including human are constituted of cells with different function and identity. Cell identity depends on gene expression programs. Single nucleotide polymorphisms in super-enhancers have recently been associated with a wide range of human diseases (Hnisz et al., 2013, Cell, 155, 934-47), suggesting the destabilization of cell identity causes disease. However, the mechanisms that suppress cell-type gene expression programs are poorly understood. Here we show that serum response factor (Srf), a transcription factor that is activated by various extracellular stimuli such as extracellular matrix stiffness, can suppress cell-type gene expressions. Depletion of β-actin increased the nuclear localization of Mkl1, a cofactor of Srf, resulting in the activation of Srf. Misactivation of Srf, which can be achieved by the suppression of *Actb* (β-actin-encoding gene) as well as overexpression of *Mkl1* or *Srf* downregulated cell-type gene expressions. Transgenic mice overexpressing *Srf* exhibited various symptoms associated with cell identity loss. Moreover, analyses of publicly available microarray data of human diseases suggested that the misactivation of Srf is also associated with various human diseases. Srf could be a possible target molecule for therapy of various diseases.

Symposium

1-S07-3

Strategy based on knowledge obtained from patients and mice models for development of novel therapy against hereditary sensorineural hearing loss (HSNHL) causing from impaired actin turnover

Takehiko Ueyama[1]

Non-syndromic HSNHL occurs 1 in 1000 live births. Newborn hearing screening had been introduced around 2000; however, no effective therapy for SNHL has been developed. About 100 genes associated with non-syndromic HSNHL has been discovered: about 30 of these encode proteins associate with actin. We are intensively studying about Rho-family GTPases, which are key regulators for actin structures. During the study of hair cell-specific Cdc42-KO mice, we found that the activated RhoA signaling is one of causes of progressive SNHL in Cdc42-KO mice. RhoA is a regulator of DIA1, which is a key molecule in straight actin elongation and the responsible gene of the 1st type of autosomal dominant non-syndromic SNHL, DFNA1. We have discovered novel DFNA1 patients and clarified that DFNA1 is caused from constitutively active mutants of DIA1, in which the auto-inhibitory interaction is disrupted. Besides, patients with CDC42 mutants showing HSNHL were reported from Japan in 2015, and they are categorized into Takenouchi-Kosaki syndrome.
I will present recent advances of our studies regarding SNHL and our trial for development of novel drugs against SNHL.

Symposium

1-S07-4

The actin cytoskeleton in cardiomyocytes: implications for cardiac function

Ryu Takeya[1]

[1]Dept. Pharmacol., Fac. Med., Univ. of Miyazaki

Contraction of cardiac muscle is caused by periodic sliding of an array of thin actin filaments into a lattice of thick myosin filaments in the sarcomere, the contractile unit of striated muscles. In actively contracting cardiomyocytes, thin filaments exhibit continuous exchange of actin subunits at their ends, although underlying mechanisms are not well understood. Fhod3, a cardiac member of the formin family proteins, is a likely candidate for a key regulator of actin dynamics in cardiomyocytes. We have shown that Fhod3 is required for cardiac development and the maintenance of the normal contractile function of the heart. Here we show a direct molecular link between Fhod3 and cMyBP-C, a thick myosin filament-associated protein that modulates myocardial contraction via cross-bridge arrangement. The direct interaction between Fhod3 and cMyBP-C appears to serve to control the Fhod3-mediated actin turnover in a manner that depends on cross-bridge arrangement. Indeed, overexpression of Fhod3 in the absence of cMyBP-C adversely affected cardiac function with a defect of sarcomere integrity. We will discuss the underlying mechanism and also discuss the possibility that targeted disruption of this cross-talk machinery leads to an artificial modulation of cardiac contractility.

Symposium

1-S08-1

Clinical study of GIRK channel inhibitors as candidate medicines for drug dependence

Kazutaka Ikeda[1]

[1]Addictive Substance Project, Tokyo Metropolitan Institute of Medical Science

G-protein activated inwardly rectifying potassium (GIRK, Kir3) channel is one of the effectors in signal pathways from ethanol, opioid, dopamine, and other addictive substances. We found associations between genetic polymorphisms in the GIRK subunit genes and sensitivity to addictive substances in mice and humans. We found that fluoxetine and paroxetine, selective serotonin reuptake inhibitors (SSRIs), but not fluvoxamine, another SSRI, inhibited GIRK channels in vitro and reduced preference for methamphetamine in mice. In addition, we found that ifenprodil, a widely used drug for dizziness, also inhibited GIRK channels in vitro. Another research group has shown that ifenprodil reduced preference for addictive substances using rodents. Furthermore, we found that relapse rate and relapse risk scores were lower in alcoholics who were treated with GIRK inhibitors. We also demonstrated an inhibitory effect of ifenprodil on alcohol use in patients with alcohol dependence in a prospective, randomized, controlled, rater-blinded study. These results suggest that GIRK channels are important molecules in the reward system and candidate targets for pharmacotherapy of drug dependence.

Symposium

1-S08-2

New three molecule regulate the dependece by methanphetamine

Atsumi Nitta[1]

[1]Dept. Neuropharmacol. & Pharma Ther., Grad. Sch. Med. Pharm. Univ of Toyama

We found and study new three molecules related methamphetamine dependence, Shati/Nat8L, Piccolo and TMEM168. Accumbal Shati rescue the dependence-induced by methamphetamine via mGluR3, and dopaminergic deficiency. Reduce of Piccolo can inhibit methamphetamine dependence via GABA regulation. TMEM168 also inhibit the methamphetamine dependence via osteopontin pathways. We found some pathway to establish drug dependence. We expected them for the new medical target for the drug dependence.
Recently, we attempt to find these molecular regulation system to develop new medical tool. Here we would like to recent our results

Symposium

1-S08-3

Neural mechanisms underlying stress-induced enhancement of cocaine rewarding properties

Katsuyuki Kaneda[1]

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Stress potentiates rewarding properties of cocaine. To elucidate neural mechanisms underlying this effect of stress, we developed an experimental paradigm combining cocaine-induced conditioned place preference (CPP) with a restraint stress. Acute restraint stress exposure immediately before posttest significantly increased cocaine CPP scores. It has been suggested that the extracellular noradrenaline (NA) level is increased by stress in the laterodorsal tegmental nucleus (LDT), which sends cholinergic projections to dopamine (DA) neurons in the ventral tegmental area (VTA), and in the medial prefrontal cortex (mPFC), which receives DA input from the VTA. Thus, we investigated the roles of NA in these brain regions. Intra-LDT injection of an α2 adrenoceptor antagonist or intra-mPFC injection of an α1 adrenoceptor antagonist attenuated the stress-induced enhancement of cocaine CPP. *In vitro* whole-cell recordings revealed that α2 adrenoceptor stimulation reduced GABAergic inputs to LDT cholinergic neurons and that α1 adrenoceptor stimulation directly excited mPFC pyramidal neurons. These findings suggest that stress-induced increases in neuronal activity of the LDT and mPFC contribute to the enhancement of rewarding properties of cocaine.

Symposium

1-S08-4

Pharmacological profile of nalmefene for reducing alcohol consumption

Yuta Ohgi[1]

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Nalmefene, an opioid system modulator, is approved in the EU and other countries for as-needed use to reduce alcohol consumption in patients with alcohol dependence. Accumulating evidence indicates that the endogenous opioid system has important roles in alcohol dependence. Alcohol stimulates the release of endogenous opioid peptides such as β-endorphin and dynorphin in the brain. β-endorphin activates μ-opioid receptor leading to euphoric mood and positive reinforcement, while dynorphin activates κ-opioid receptor leading to dysphoric mood and negative reinforcement. These euphoric/dysphoric mood and reinforcement effects via endogenous opioid systems are suggested to be implicated in repeated alcohol intake in patients with alcohol dependence.

Nalmefene acts as an antagonist at μ- and δ-opioid receptor and a partial agonist at κ-opioid receptor. Preclinical studies have shown that nalmefene reduced the alcohol intake in alcohol preference rats. In clinical trials, as-needed use of nalmefene with psychosocial support reduced the number of heavy-drinking days and total alcohol consumption. These results suggest that nalmefene modulates the alcohol-induced euphoric/dysphoric mood via opioid system and thereby contribute to reduction in alcohol consumption in patients with alcohol dependence.

In this symposium, we will discuss the implications of opioid system in alcohol dependence and pharmacological profiles of nalmefene in preclinical and clinical studies.

Symposium

1-S09-1

Feeding-related GPCR signaling through the neuronal primary cilium

Yumiko Saito[1], Yuki Kobayashi[1]

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Non-motile primary cilia are sensory organelles that present in most vertebrate cell types. Its localization within tissue architecture and a growing list of cilia-localized receptors, in particular a limited set of G-protein-coupled receptors (GPCRs), determine a host of crucial physiologies, which are disrupted in human ciliopathies. Melanin-concentrating hormone (MCH) is a cyclic neuropeptide exerting its action through two GPCRs, MCHR1 and MCHR2. The extensive progress using genetic and pharmacological approaches has confirmed that the MCH-MCHR1 system is involved in feeding and possibly emotional processing. We recently found the that MCH signaling through a Gi/o-Akt pathway induces cilia length shortening in ciliary MCHR1-expressing RPE1 cells without no cell cycle progression. This is the first example of effective-neuropeptide-induced cilia length reduction. Here, we discuss our recent progress in the characterization of signaling components that cause cilia shortening via ciliary MCHR1 localized in neuron. Short cilia phenotypes have been associated with various metabolic disorders. Thus, our study has highlighted the unique signaling environment of the primary cilium, in which the design allows for organized signaling in neuronal network toward feeding.

Symposium

1-S09-2
Molecular basis of intraflagellar transport and ciliopathies

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Cilia are microtubule-based appendages that project from the surfaces of various eukaryotic cells and play critical roles in sensing extracellular stimuli and transducing developmental signals. Defects in the assembly and functions of cilia result in a variety of congenital disorders, which are collectively called the ciliopathies.
The bidirectional trafficking of ciliary proteins along the microtubule-based axoneme is mediated by the intraflagellar transport (IFT) machinery, which contains the two large multisubunit complexes IFT-A and IFT-B. Anterograde protein trafficking from the base to the tip of cilia is mediated by the IFT-B complex driven by kinesin-2 motor proteins, whereas retrograde trafficking is mediated by the IFT-A complex driven by the dynein-2 complex.
I will introduce the architecture and function of IFT machinery revealed by utilizing the visible immunoprecipitation (VIP) assay, which we recently developed as a simple and flexible strategy for visually detecting protein-protein interactions, and CRISPR/Cas9 genome editing.

Symposium

1-S09-3

Visualization of primary cilia topography using scanning probe microscopy

Yasufumi Takahashi[1], Yuanshu Zhou[1], Masaki Saito[2], Takeshi Fukuma[1]

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Primary cilia are hair-like sensory organelles whose dimensions and location vary with cell type and culture condition. Herein, we employed scanning ion conductance microscopy (SICM) to visualize the topography of primary cilia from different cell types. By combining SICM with fluorescence imaging, we successfully distinguished between surface cilia that project outward from the cell surface and subsurface cilia that are trapped below it. The nanoscale structure of the ciliary pocket, which cannot be easily identified using a confocal fluorescence microscope, was observed in SICM images. Furthermore, we developed a topographic reconstruction method using current-distance profiles to evaluate the relationship between set point and topographic image and found that a low set point is important for detecting the true topography of a primary cilium using hopping mode SICM.

Symposium

1-S09-4
Mechanisms of cell proliferation through primary cilium

Masaki Saito[1], Wataru Otsu[2], Ching-Hwa Sung[2]

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Primary cilium is a nonmotile sensory organelle that possesses selective membrane receptors. The cilium is dynamically regulated in a cell cycle-dependent manner; it is displayed at the G_0/G_1 phase of many cell types, including neural progenitor cells, and resorbed prior to the S phase. The ciliary dynamics has pivotal roles in development of many tissues/organs. However, the molecular mechanism how the cilium controls cell proliferation remains largely unknown. We found that IGF-1 was one of the growth factors that promoted proliferation of neural progenitors. Tctex-1, a light chain of cytoplasmic dynein that plays a key role in ciliary resorption, can be free from dynein complex when it is phosphorylated at Thr 94. We also found that phospho-Tctex-1 was enriched at the ciliary base in the cells. Molecular approaches analyzed in an immortalized retinal pigment epithelial cell line revealed the physical and functional interaction of phospho-Tctex-1 with the regulators of actin dynamics such as annexin A2, Arp2/3 complex and Cdc42, which promoted branched actin polymerization and dynamin- and clathrin-dependent endocytosis at peri-ciliary region. Our study demonstrated that these mechanisms collectively regulate ciliary resorption and proliferation of neural progenitors.

Symposium

1-S10-1

Immune regulation, inflammation, and vaccine adjuvant by using lymphoid tissue-resident commensal bacteria

Jun Kunisawa[1]

[1]Laboratory of Vaccine Materials and Laboratory of Gut Environmental System, National Institutes of Biomedical Innovation, Health and Nutrition

Intestinal commensal bacteria is now recognized to be an important element in the control of the development and function of the host immune system, including the production of secretory IgA and differentiation of specific T cell populations. Although many studies mainly focused on the commensal bacteria in the intestinal lumen or mucus layers, genome-based bacterial analysis using intestinal tissue allowed us to identify *Alcaligenes* as symbiotic resident bacteria of Peyer's patches (PPs), a major gut-associated lymphoid tissue in the small intestine, which is regulated by type 3 innate lymphoid cells (ILC3) . Our subsequent study showed that *Alcaligenes* have a greater ability to survive in dendritic cells (DCs) and modulate the production of cytokines such as IL-1b, IL-6, IL-10, IL-12p40, and IL23 from DCs. We recently found that lipopolysaccharide (LPS) of *Alcaligenes* acts as a weak TLR4 agonist and thus creates a homeostatic inflammatory condition that includes IgA responses in PPs without the excessive pathological inflammation These findings allowed us to apply *Alcaligenes* LPS could be used as a safe and effective vaccine adjuvant.

Symposium

1-S10-2

Quantitative evaluation of kinase activities and transcriptional regulation using mathematical model

Mariko Okada[1]

[1]Laboratory of Cell Systems Institute for Protein Research, Osaka University

Cells respond to external stimuli and eventually make the decision for survival, growth and differentiation. In this process, outcomes of the kinase activities of signal transduction pathways and transcription factors often show dynamically rich, highly quantitative behaviors over time. This quantitative response is, however, eventually converted into a qualitative response and a binary decision (eg. survival or cell death), at the stage of cell decision. A binary decision of a cell is made based on the combined activities of multiple molecules. However, the molecular mechanism of threshold setting of the responses in each cellular context is still unknown. To reveal this mechanism, we analyze the experimental data of signaling and multi-Omics using a mathematical model and extract logical rules in the cell decision mechanism and the target molecules (eg. marker molecules) that define the cellular threshold.

Our analysis, using NF-kB and ErbB receptor signaling as model systems, indicates that the high-order inter-molecular formation in signaling pathways and epigenetic regulation plays an important role for binary activation of gene expression, and this mechanism might act as a threshold setting for cell regulation. I will introduce our modeling approach for NF-kB signaling pathway, single cell transcriptome and epigenetics.

Symposium

1-S10-3

Gene therapy approaches for sepsis with decoy ol: igodeoxynucleotides targeting different transcription factors

Kengo Tomita[1], Yuichi Hattori[1]

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Sepsis, a syndrome that occurs when microbial invasion induces systemic illness, is one of the most common reasons for critical ill patients to be admitted to an intensive care unit and, despite advances in overall medical care, it represents a major clinical problem and remains the leading cause of death in the critically ill patient population. A major hurdle in the clinical management of patients suffering from the sepsis syndrome is the lack of the effective treatment. Thus, the important goal in critical care medicine is to find out significant therapeutic strategies that would impact favorably on patient outcome. Gene therapy can be considered as one of the most promising novel therapeutic approaches for nasty disorders. Since sepsis can be characterized by the induction of multiple genes and their products, sepsis may be regarded as a gene-related disorder. A number of transcription factors, including NF-kB, AP-1 and STAT3, are profoundly activated during sepsis, and may play a pivotal role in the pathophysiology of sepsis. The decoy strategy has been developed as a useful tool for the involvement of those transcription factors in disease pathology. The decoy oligodeoxynucleotides (ODNs) can specifically compete for the binding elements of those transcription factors, thereby blocking their actions. We have devised the potential usefulness of different transcription factor ODNs for gene therapy of sepsis. Our results suggest that the development of decoy ODN techniques for those transcription factors may provide new perspectives on revolutionary gene-therapy approaches for the fight against sepsis.

Symposium

1-S10-4
Dynamics of host chromatin 3D response to virus infection

Yumiko Imai[1], Yu Ichida[1], Masami Shiimori[1]

[1]Laboratory of Regulation for Intractable Infectious Diseases, National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN)

Influenza viruses cause worldwide epidemics and pandemics, and sometimes trigger critical illness especially among humans with high risk factors, including obesity, diabetes, and cancers, of which population is increasing with the aging of society. To date no effective preemptive medicine or treatment of severe influenza has been established. Influenza virus is a single stranded RNA virus, and transcription and replication of the virus genome occur in the nucleus. Since viral infection is generally associated with virus-driven hijack of the host cellular machineries, influenza virus may utilize and/or affect nuclear system. Recent high-resolution chromatin interaction maps using chromosome conformation capture (3C) techniques such as 4C and Hi-C have defined units of chromatin that are 3D, termed topologically associated domains (TADs). In the present study, using 4C-seq and ChIP-seq we examined how host chromatin 3D structure and epigenetic modification were changed to influenza virus infection, and how they were involved in the pathology of severe influenza. Our data suggest that host chromatin 3D dynamics could be a novel target of prevention and/or treatment for severe influenza virus infection.

Symposium

2-S11-1

Prevention of chemotherapy-induced peripheral neuropathy by targeting HMGB1

Atsufumi Kawabata[1]

[1]Lab. of Pharmacol & Pathophysiol, Fac of Pharm, Kindai Univ

High mobility group box 1 (HMGB1), a damage-associated molecular pattern (DAMP) protein, is considered to play a role in chemotherapy-induced peripheral neuropathy (CIPN), since an anti-HMGB1-neutralizing antibody prevents CIPN in rodents. Thrombomodulin alfa (TMa), a recombinant human soluble thrombomodulin, is capable of promoting thrombin-dependent degradation of HMGB1. TMa inhibits intraplantar HMGB1-induced allodynia and prevents CIPN caused by distinct chemotherapeutics in rodents. Inhibitors of thrombin, vitamin K or factor Xa attenuate or abolish the preventive effect of TMa on oxaliplatin-induced peripheral neuropathy (OIPN). Repeated administration of those agents aggravates the OIPN and elevates plasma HMGB1 levels. Our retrospective cohort study shows that hepatic dysfunction, possibly due to oxaliplatin therapy, is a risk factor for severe OIPN. Our preclinical study demonstrates that hepatic damage promotes OIPN most probably by promoting HMGB1 release in mice. Together, our data suggest that TMa is available for prevention of CIPN, which requires special attention on co-administration of anti-coagulants, and that careful monitoring of hepatic function is useful to predict severe OIPN.

Symposium

2-S11-2

Identification of biomarkers for taxanes-induced peripheral neuropathy and search for new therapies by drag-repositioning

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Chemotherapy-induced peripheral neuropathy (CIPN) is a frequent side effect of taxanes. Because of the as yet poor understanding of the mechanism underlying CIPN pathogenesis, there is no indicator for objective diagnosis like a biomarker. In addition, treatment options for CIPN remain largely unsatisfactory. Previous our study demonstrated that paclitaxel preferentially impair myelin-forming Schwann cells, and consequently induce dedifferentiation and demyelination of Schwann cells. Recently, in a paclitaxel CIPN mouse model, we found that an inflammatory factor is released from dedifferentiated Schwann cells in the mouse sciatic nerve into the blood, highly correlated with the on-set of pain hypersensitivity. In this presentation, I will introduce the usefulness of this inflammatory factor as a biomarker for the progression of CIPN.
On the other hand, considering our previous findings, it seems that some drugs, which induce differentiation of Schwann cells and supply newly formed mature Schwann cells at sites of demyelinated lesions, may be a new therapy for CIPN. Now, I am promoting therapeutic drug screening for CIPN based on this concept, and I will talk about a part of our results in this presentation.

Symposium

2-S11-3

Effects of Cryotherapy on Chemotherapy-Induced Peripheral Neuropathy: Self-Controlled Clinical Trial

Akiko Hanai[1]

[1]National Cancer Center

Chemotherapy-induced peripheral neuropathy (CIPN) is a frequent and disabling side effect of cancer treatment. We evaluated the preventive effects of cryotherapy in a self-controlled trial.
Forty breast cancer patients who were planned to undergo weekly paclitaxel treatments (80 mg/m^2 for 1 hour) with a cumulative dose of at least 960 mg/m^2 were enrolled. Each patient wore frozen gloves and socks on the dominant hand and foot, and the non-dominant side acted as the untreated control. CIPN symptoms were assessed by tactile sensitivity, thermal sensitivity, performance speed, and Patient Neuropathy Questionnaire. We defined tactile sensitivity, patients-blinded test, as the primary outcome. We concluded that cryotherapy is useful for preventing both the objective and subjective symptoms of CIPN and resultant dysfunction.
There were other reports about cold intolerance or frostbites due to cryotherapy. In our trial, nonwoven fabric covers were applied to alleviate the discomfort if the patients complained of cold pain. The subgroup analysis indicated that this intervention did not interfere with the effects of the cryotherapy. Further discussion to establish standard cryotherapy with safe and adaptable settings is needed.

Symposium

2-S12-1
Neuroinflammation underlying pathogenesis of Alzheimer's disease

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Alzheimer's disease (AD) is the most common type of neurodegenerative disorder in the world. Although both amyloid beta peptide deposition and neurofibrillary tangle (NFT) formation in the AD brain have been established as pathological hallmarks of the disease, many other factors contribute in a complex manner to the pathogenesis of AD. Longitudinal pathophysiological processes cause patients' brains to exist in a state of chronic neuroinflammation; additionally, reactive glial cells contribute to AD pathogenesis. However, the detailed molecular and cellular mechanisms underlying this pathogenesis are still unclear. Such disease complexities make it difficult for the pathogenesis of AD to be understood, and impede the development of effective therapeutic strategies to combat the disease. Relevant AD animal models are thus likely to serve as a key resource to overcome many of these issues. In this symposium, I will introduce current situation of research and share perspectives for understanding glial pathophysiology.

Symposium

2-S12-2

Age-related decline of neural activities in the motor cortex is ameliorated by coenzyme Q_{10}.

Ritsuko Inoue[1], Mayumi Takahashi[2], Masami Miura[1]

[1]Res Team for Aging Neurosci., Tokyo Met Inst of Gerontology, [2]Res Team for Functional Biogerontology, Tokyo Met Inst of Gerontology

Not only neurological disease but also physiological aging affects brain function. Takahashi et al (2016) found that histological and motor functional alterations were associated with age-related decline of brain mitochondrial function. Aged mice (15-month-old) displayed significant reductions in mitochondrial oxygen consumption rate, coenzyme Q (CoQ) content, vesicular glutamate transporter 1 level in the motor cortex, and motor function compared to young mice (6-month-old). CoQ is a coenzyme, and present in the mitochondria. It is an electron transporter in the respiratory chain involved in ATP production. However, age-related electrophysiological impairments of the motor cortex were poorly understood. In this talk, I will describe how physiological aging affects electrophysiological activities in the motor cortex of mice, and present data to show how exogenous CoQ treatment affects the age-related alteration in the aged mice. I will also discuss the influence of exogenous CoQ treatment on the age-related decline of electrophysiological activities in the motor cortex. Takahashi and our studies suggested that the age-related alterations were ameliorated by exogenous CoQ treatment. Although the relation between central nervous system and age-related motor decline remain to be elucidated, our results may serve as the basis for developing therapy of age-related motor impairment.

Symposium

2-S12-3
Breakthrough that links drug development of Alzheimer's disease

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Memantine ameliorates progressive symptomes in Alzheimer's disease (AD) through moderate inhibition of *N*-methyl-*D*-aspartate receptors (NMDARs). Here we report that a novel target of mementine, ATP-sensitive K^+ (K_{ATP}) channels are implicated in memory improvement. K_{ATP} channels Kir6.1 or Kir6.2 are composed with sulfonylurea receptors (SURs), which are distributed both in peripheral tissues and central nervous system. We confirmed that memantine improves both memory impairment and perturbed NMDAR-dependent LTP in APP23 mouse hippocampus. Unexpectedly, memantine in vivo increased CaMKII activity in APP23 hippocampus, and memantine-induced enhancement of hippocampal LTP and CaMKII activity was in vitro abolished by treatment with pinacidil, a specific opener of K_{ATP} channels. We therefore confirmed that memantine inhibits K_{ATP} channels Kir6.1 and Kir6.2 and elevates intracellular Ca^{2+} concentrations by inhibition of Kir6.1 or Kir6.2. Kir6.2 was preferentially expressed in the postsynaptic regions, whereas Kir6.1 was predominant in mouse hippocampal neuron dendrites. Finally, we confirmed that Kir6.2 heterozygous mutant mice exhibit severe memory deficits and hippocampal LTP impairment that could not be rescued by memantine administration. Taken together, we propose a novel strategy that memantine inhibits Kirs 6.2/6.1 activities, thereby improving memory impairment in AD patients.

Symposium

2-S12-4

Possible Involvement ROS-Dependent Inhibition of Protein S-nitrosylation in Physiological Aging of Mouse Cerebellum

Sho Kakizawa[1]

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Reactive oxygen species (ROS) is considered as one of the main factors inducing physiological aging. However, molecular mechanisms how ROS induce physiological and pathological aging have not been fully understood.

In the cerebellar parallel fiber (PF)-to-Purkinje cell (PC) synapse (PF synapse), nitric oxide (NO)-dependent long-term potentiation (PF-LTP) is characterized. Previous studies indicated that the PF-LTP is dependent on S-nitrosylation of type I ryanodine receptor (RyR1), an intracellular Ca^{2+}-release channel, and the resulting novel type of Ca^{2+}-release, nitric oxide-induced Ca^{2+}-release (NICR) in cerebellar PCs.

Thiol groups in cysteine residue are the target of S-nitrosylation of proteins by NO as well as the target of disulfidation (disulfide-bond formation) by ROS. Thus, it is highly possible that protein disulfidation blocks the induction of S-nitrosylation and the resulting S-nitrosylation-dependent biological events, such as PF-LTP.

In this symposium, I will introduce our recent studies designated to test this hypothesis. In the cerebellar slices pretreated with ROS, S-nitrosylation of RyR1, NICR and PF-LTP were impaired. Furthermore, in the cerebellar slices from aged mice (about 2-years old), RyR1 S-nitrosylation, NICR and PF-LTP were again inhibited. These results support the hypothesis, and also suggest that endogenous ROS possibly induce physiological aging through the inhibition of S-nitrosylation in old animals.

Symposium

2-S13-1

The Roles of hypoxia signaling in sterile inflammation and tissue remodeling

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Hypoxia is a condition in which the tissue is deprived of adequate oxygen supply. It occurs in several cardiovascular disorders, such as cardiac hypertrophy and myocardial ischemia, and accelerates the inflammatory processes. While each cell exerts its own responses to hypoxia, most of them are mainly mediated through the transcription factor, hypoxia inducible factor-1a (HIF-1a) and HIF-2a. Macrophages are key mediators of inflammation, and can be broadly classified as M1 (pro-inflammatory) and M2 (anti-inflammatory) type. We found that HIF-1a and HIF-2a is specifically expressed in M1 and M2 macrophages respectively. The balance between HIF-1a and HIF-2a, termed as HIF-a switching, regulates macrophage activation and its resolution. We further examined the roles of macrophage hypoxia signaling in cardiac remodeling using mice transverse aortic constriction model, and found that M1 macrophages accumulate into the hypoxic area though a HIF -1a dependent manner. As an underlying molecular mechanism, we discovered that HIF-1a mediated glycolytic reprograming is critically required in macrophage migration potential. Importantly, LV hypertrophy and cardiac fibrosis were more prominent while systolic function was impaired in HIF -1a knockout mice. These results demonstrate a novel functional link between hypoxia activated cardiac macrophage and cardiovascular remodeling.

Symposium

2-S13-2

The effect of PHD inhibitor on tumor microenvironment and tumor immune response

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The blood vessel is important tissue structures to deliver oxygen, nutrition and so on. An abnormal blood vessel formation is a common feature of tumor tissue that were characterized by hyper-permeability, irregular vascularization, immature vessels and intravasation. Therefore, tumor tissue is exposed to low oxygen nutrition depletion and low pH due to hypoperfusion and elevated interstitial pressure. These environments are one of the reasons for chemo- and radio-resistance. Previously, we reported that prolyl hydroxylase (PHD) inhibitor induced tumor blood vessel normalization and improved tumor microenvironment in tumor mouse model. However, effects of PHD inhibitor on tumor progression is controversial. Enhanced hypoxia inducible factors (HIFs) signaling in cancer cells act to promote cancer proliferation and metastases. On the other hand, increasing of HIFs signaling in immune cells may lead to activate inflammation and elicit anti-tumor effect. In this session, we will talk about our study how PHD inhibitor improved tumor microenvironment and focused on tumor infiltrate immune cells were phenotypic alteration after PHD inhibitor treatment in mouse model. We will also discuss about usefulness of PHD inhibitor for anti-cancer therapy.

Symposium

2-S13-3

Targeting hypoxic response machinery for the treatment of critical diseases ～ Clinical application of PHD inhibitors ～

Yoji Andrew Minamishima[1]

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Loss of prolyl hydroxylase 2 (**PHD2**) activates hypoxia-inducible factor (**HIF**)-dependent **hypoxic response** including enhanced **anaerobic glycolysis**, which releases great amount of **lactate** from cells into circulation. However, surprisingly, systemic activation of hypoxic response by PHD2 inhibition in mice did not lead to hyperlactatemia. This serendipitous phenomenon led us to hypothesize that the activated hypoxic response enhances **Cori cycle**, the lactate-glucose carbon recycling system between muscle and liver, and then reduces circulating lactate level. Here we show that liver-specific inactivation of PHD2 improves the survival of **lactic acidosis** by activating Cori cycle in the liver, and pharmaceutical inhibition of **PHDs** also improves the survival of lethal lactic acidosis induced by endotoxin shock. Lactic acidosis is also known to be induced by **metformin**, which is a popular therapeutic for type 2 diabetes mellitus and also has anti-cancer and anti-aging properties but is contraindicated in individuals with **chronic kidney disease (CKD)** due to the risk of **metformin-associated lactic acidosis (MALA)**. We also report that treatment with a **PHD-inhibitor** *per os* significantly improves the survival rate of MALA in CKD mice. Our findings would provide a new concept that the **oxygen sensor** PHDs serve as new therapeutic targets for the treatment of endotoxin shock-induced lactic acidosis or MALA. The application of PHD-inhibitor as the rescue agent for the renal anemia or myocardial infarction will be also discussed.

Symposium

2-S13-4
Multiple consequences of HIF activation in CKD

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In chronic kidney disease (CKD), tubulointerstitial hypoxia is regarded as a final common pathway leading to end-stage kidney disease. Insufficient oxygenation negatively influences the balance between injury and repair in tubular epithelial cells.
Studies on erythropoietin (EPO) transcription led to the identification of hypoxia inducible factors (HIFs) and their key regulators, prolyl hydroxylases (PHDs). Based on these, several small molecule PHD inhibitors are developed for the treatment of anemia in CKD, which are currently in phase II/III clinical trials. Studies so far demonstrate successful increases in hemoglobin levels by raising plasma EPO levels and optimizing iron utilization.
Application of PHD inhibitors has several potential implications beyond anemia treatment, and there is a promising view that activation of the HIF signaling might protect the ischemic kidney from injury. This concept is extensively tested in multiple acute kidney injury models, but knowledge is limited in the context of CKD. Some studies demonstrate the protective effects of ameliorating inflammation and reducing oxidative stress. In human clinical studies, some of the PHD inhibitors exhibit the additional advantage in terms of glucose and lipid metabolism, which may be beneficial for the treatment of metabolic kidney disorders. On the other hand, negative consequences of sustained HIF activation are also reported, including renal fibrosis and aggravation of polycystic kidney disease. Renal consequences are likely determined by multiple systemic effects of PHD inhibition and may thus differ depending on the clinical context and the pathological stages.

Symposium

2-S14-1

Cell surface pH imaging using a membrane-anchored ratiometric fluorescence probe: Poly(ethylene glycol)-phospholipid as membrane anchor to investigate the juxtamembrane environment

Ryuichi Ohgaki[1], Yuji Teramura[2], Daichi Hayashi[1], Shushi Nagamori[1,3], Madoka Takai[2], Yoshikatsu Kanai[1]

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Various physiological and pathological processes are accompanied with the alteration of extracellular local pH. Accordingly, there has been a strong demands for the development of methods to analyze the cell surface pH. We established a novel method of *in vitro* cell surface pH imaging by using a membrane-anchored pH probe, poly(ethylene glycol)-phospholipid conjugated with fluorescein isothiocyanate (FITC-PEG-lipid). When added into the cell culture medium, FITC-PEG-lipid is spontaneously inserted into the plasma membrane via its phospholipid moiety, and retained at the extracellular surface. The ratiometric readout of its fluorescence was unique to the extracellular pH in the range of weakly alkaline and acidic pH. Our study demonstrated that FITC-PEG-lipid is useful as a sensitive and reversible cell-surface-anchored pH probe. The simple cell-surface labeling procedure of FITC-PEG-lipid is advantageous especially when considering its application to high-throughput *in vitro* assay. Furthermore, PEG-lipid holds a great potential as the membrane anchor of various analytical probes to approach the juxtamembrane environments.

Symposium

2-S14-2

Negative regulation of gastric proton pump by desialylation suggested by fluorescent imaging with the sialic acid-specific nanoprobe

Takuto Fujii[1], Takahiro Shimizu[1], Keiichiro Kushiro[2], Hiroshi Takeshima[3], Madoka Takai[2], Hideki Sakai[1]

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Gastric proton pump (H^+,K^+-ATPase) consists of two subunits, a catalytic α-subunit and a glycosylated β-subunit (βHK). So far, properties of the individual carbohydrate residues of βHK have been unclear. Here, we succeeded in visualizing the sialylation and desialylation dynamics of βHK using a fluorescence bioimaging nanoprobe that specifically detects sialic acids. The fluorescence of the probe was observed at the cell surface of H^+,K^+-ATPase-expressing living LLC-PK1 cells but not in non-expressing cells. The fluorescence and H^+,K^+-ATPase activity in the cells were significantly decreased by sialidase and acidic solution. In gastric mucosa of rats and hogs, the fluorescence of the probe was observed in the samples treated by famotidine, an H_2 blocker, but not by histamine, an acid secretagogue. H^+,K^+-ATPase activity in the famotidine-treated samples was significantly higher than the histamine-treated samples. These famotidine-induced effects were weakened by sialidase. Our studies using the nanoprobe uncover a novel negative-feedback mechanism of H^+,K^+-ATPase in which sialic acids of βHK positively regulates H^+,K^+-ATPase activity, and acidic pH decreases the pump activity by cleaving sialic acids of βHK.

Symposium

2-S14-3

Nanoscale imaging of extracellular microenvironment using scanning ion conductance microscopy

Yasufumi Takahashi[1], Yuanshu Zhou[1], Takuto Fujii[2], Hideki Sakai[2], Takeshi Fukuma[1]

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Local metabolite and ionic strength are important factors for maintaining the functions of living cells. We have developed micro-nanoscale electrode and electrochemical sensor based scanning probe microscopy to measure the spatial electrochemical metabolite and ion concentration profile near the sample surface with nanoscale resolution. Scanning electrochemical microscopy (SECM) uses an ultramicroelectrode as a probe for detecting electroactive chemical species (oxygen, ATP, and reactive oxygen species (ROS), and neurotransmitter). SECM has been recognized as an effective tool for investigating micrometer-scale local chemical flux. Miniaturization of the electrode is an important factor for improving SECM resolution. Electrode–sample distance control is also an important factor for measuring fast chemical flux and improving SECM resolution. Distance control by ion current feedback is a promising way for the non-contact investigation of soft materials, SECM–scanning ion-conductance microscopy (SICM) has been used in a hybrid system to improve SECM resolution by controlling the electrode probe and sample distance in solutions without direct contact. SICM is also useful for detecting the ion concentration profile and charge measurement in a solution. We measured the 3D chemical and ion current using SECM–SICM. In this presentation, we report the SICM topography images of gastric surface mucous cell lines (GSM06), which produce periodic acid-schiff and concanavalin A positive glycoproteins. To visualize the damage process of the mucosal layer, we added ethanol to GSM06 cells and imaged the topography change using SICM. We also performed topography and electrochemical simultaneous imaging using SICM-SECM to identify the mucosal layer without labelling.

Symposium

2-S14-4

Development of *in vivo* drug sensing system with needle-type diamond microelectrode.

Genki Ogata[1], Kai Asai[2], Yamato Sano[3], Seishirou Sawamura[1], Madoka Takai[4], Hiroyuki Kusuhara[3], Yasuaki Einaga[2], Hiroshi Hibino[1]

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Continuous and real-time measurement of local concentrations of systemically administered drugs *in vivo* must be crucial for pharmacological studies. Nevertheless, conventional methods require considerable samples quantity and have poor sampling rates. Additionally, they cannot determine how drug kinetics correlates with target function over time. Here, we describe a system with two different sensors. One is a needle-type microsensor composed of boron-doped diamond with a tip of □40 μm in diameter, and the other is a glass microelectrode. We first tested bumetanide. This diuretic can induce deafness. In the guinea-pig cochlea injected intravenously with bumetanide, the changes of the drug concentration and the extracellular potential underlying hearing were simultaneously measured in real time. We further examined an antiepileptic drug lamotrigine in the rat brain, and tracked its kinetics and at the same time the local field potentials representing neuronal activity. The action of the anticancer reagent doxorubicin was also monitored in the cochlea. This microsensing system may be applied to analyze pharmacokinetics and pharmacodynamics of various drugs at local sites *in vivo*, and contribute to promoting the pharmacological researches.

Symposium

2-S15-1
Chronopharmacological strategy for treatment of malignant tumors

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With our social change toward a 24-hour society, a substantial proportion (ca. 27%) of workers is engaged in shift-work schedules. However, epidemiological studies have indicated that such shift-work schedules increase the risk of obesity, cancers, diabetes, and cardiovascular diseases. Circadian clock-related disorders are now prevalent in our society. In mammals, circadian rhythms in physiological function are regulated by a molecular oscillator consisting of circadian clock genes. Disruption of cellular circadian rhythms is well-recognized to be associated with cancer development and tumorigenesis; however, the underlying mechanism is not fully understood.

Solid tumors are composed of phenotypically and functionally heterogeneous cells. Among them, highly tumorigenic cancer stem cells (CSCs) generate intermediate progenitors and terminally differentiated cells. Similar to physiological stem cells, CSCs often exhibit resistance to various chemotherapeutic drugs. Recently, we found that oncogenic transformation of circadian clock-defective cells exhibited CSC phenotype cells. Furthermore, oncogenic-transformed circadian clock-defective cells also resisted against the cytotoxicity of chemotherapeutic drugs.

In this symposium, I would like to summarize our recent findings on the underlying mechanism of development of chemoresistance in circadian clock-defective cells, and also to introduce the chronopharmacological strategy for treatment of malignant tumors.

Symposium

2-S15-2
Pharmacotherapy considering pharmacokinetic changes of vitamins

Tappei Takada[1], Yoshihide Yamanashi[1], Sayo M Ito[1], Hiroshi Suzuki[1]

Vitamin K (VK) is a fat-soluble vitamin involved in the regulation of blood coagulation. Mammals do not synthesize VK and must therefore obtain this vitamin by intestinal absorption. The molecular mechanism(s) of the VK absorption process are not clear. Based on the known role of the NPC1L1 protein in the intestinal absorption of fat-soluble compounds such as cholesterol and vitamin E, the possible uptake of VK via an NPC1L1-mediated pathway was examined. *In vitro* studies using NPC1L1-overexpressing cells and *in vivo* studies revealed that intestinal VK absorption is NPC1L1-dependent and inhibited by ezetimibe, an NPC1L1-selective inhibitor. In addition, *in vivo* pharmacological studies demonstrated that the co-administration of ezetimibe and warfarin, a VK antagonist used as an anticoagulant drug, caused a reduction in hepatic VK level and enhanced the pharmacological effect of warfarin. These adverse events caused by the co-administration were rescued by oral VK supplementation, suggesting that the drug interaction effects observed were the consequence of ezetimibe-mediated VK malabsorption. This non-idiosyncratic drug interaction mechanism was supported by clinical results showing that in the majority of warfarin-treated patients, anticoagulant activity was enhanced by co-treatment with ezetimibe. These findings suggest a novel mechanism of drug-drug interaction mediated by the alteration of the kinetics of essential vitamins. In the presentation, we are going to introduce the physiologically, pharmacologically, and clinically important topic with recent progress.

1) Takada T, *et al.*, *Science Translational Medicine.* 7:275ra23, 2015.
2) M Ito S, *et al.*, *Circulation Journal.* accepted.

Symposium

2-S15-3

Exploring biomarkers and therapeutic targets by genome copy number variation

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Research on human genetics of schizophrenia enables to discover effective targets for its diagnosis and medication because of its high heritability. Rare or de novo copy-number variations (CNVs) are likely the most significant contributors to the pathogenesis of schizophrenia. We recently found novel schizophrenia-associated CNVs including ARHGAP10. ARHGAP10 is a member of the Rho GTPase-activating protein (RhoGAP) family that contributes to organizing the actin skeleton, as well as neuronal polarization. We developed a mouse model of schizophrenia patient with both a deletion-type CNV and a single-nucleotide variation (SNV) in the RhoGAP domain of ARHGAP10 gene (ARHGAP10 mutant mice). The mutant mice showed emotional abnormality and potentiation of psychostimulant-induced hyperlocomotion. Furthermore, psychostimulant-treated ARHGAP10 mutant mice showed a marked reduction of percentage of accuracy compared with psychostimulant-treated wild-type mice as well as saline-treated ARHGAP10 mutant mice in a translatable visual discrimination task that reflects cognitive function. These findings suggest that mutations in ARHGAP10 increase the risk of schizophrenia, and Rho signaling pathway may be a potential therapeutic target to develop novel antipsychotics.

Symposium

2-S15-4

Establishing individualized medicine for intractable cancer based on clinical molecular pathogenesis －A novel predictive marker CYLD for molecular targeted therapies－

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With innovative advancements in science & technology, cancer treatment has dramatically improved by discovering molecular targeted agents. However, identifying eligible patients and predicting their therapeutic effects still remain a great challenge. Because genetic and molecular differences of tumors significantly affect therapeutic effects in clinical, establishing individualized medicine based on precise molecular pathogenesis is urgently required.

Cylindromatosis (CYLD) was originally identified as a tumor suppressor because loss of which causes a benign human tumor called cylindromatosis. Increasing clinical evidence reveals that dysfunction of CYLD by loss of its expression is believed to play key roles in diverse pathological processes in various types of tumors. Moreover, our results have shown that loss of CYLD expression not only be involved in tumor malignant transformation, but also serves as a prognostic & predictive biomarker for several tumors.

In this session, we focus on the clinical significance of CYLD and introduce our approaches toward developing a novel molecular targeted therapies. Deeper understanding of more biological feature and clinical significance of CYLD may open up novel strategies for establishing individualized cancer treatment for malignant tumors.

Symposium

2-S16-1

Constructing iPS cell-based platforms for disease modeling and drug discovery in cardiovascular fields: Phenotype analysis using self-organization and new imaging techniques

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Disease-specific iPS cells have been considered and used as platforms for disease modelinsg and drug discovery for intractable diseases. In the field of cardiovascular medicine, iPS cells have been generated from patients with heart diseases including inherited cardiomyopathy. The disease-specific iPS cells showed reproduce the certain parts of phenotype of the disease on culture dishes in in vitro systems, but the cells do not necessarily recapitulate patients clinical properties, particularly those of physiological-/pathophysiological aspects. The point should be solved to establish disease reliable platforms. The discrepancy may be attributed to the lack of developmental process during culture procedure. To settle the problems, various techniques have been attempted such as culture dishes with specific structures.
In this symposium, introducing the phenotype of disease specific iPS-cells from patients with cardiomyopathy, we will discuss what need to be done to reproduce "human hearts on culture dishes".

Symposium

2-S16-2

Safety evaluation using human induced pluripotent stem cell-derived cardiomyocytes (hiPS-CMs): Availability of evaluation system using atrial myocytes

Yayoi Honda[1]

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Since human induced pluripotent stem cell derived cardiomyocytes (hiPS-CMs) became available, its usefulness for safety evaluation aiming at improving predictability has been actively examined in many facilities. Among them, the researches focusing on drug induced ventricular arrhythmias using hiPS-CMs have been validated. On the other hand, drug related supraventricular arrhythmias including atrial fibrillation and sinus node dysfunction, which are increasing year by year in clinical, might not be detected by existing methods using hiPS-CMs because the cells being used are mainly constituted of ventricular myocytes. Currently, we are trying to establish the cardiotoxicity system using hiPS cell derived atrial myocytes, and some differences between atrial and ventricular myocytes in drug response are being acquired. In this symposium, we will introduce some evidence and discuss the usefulness of evaluation system using hiPS atrial myocytes as a detection method of supraventricular arrhythmia.

Symposium

2-S16-3

A new way to use of lights for medical application: Predication of “intracellular state” from scattering lights.

Tomonobu Watanabe[1]

[1]RIKEN Center for Biosystems Dynamics Research

Scattering light has an interesting and important feature. There are various kinds of interaction between light and molecule, and the scattered light includes internal information of the molecule. Raman scattering inheres all the vibration mode of molecular bonds composing a molecule, and second harmonic generation (SHG) light, which is one of second-order non-linear scattering light, is derived from electric polarizations in the molecule, in other words, includes structural information in protein.

While states of cell are usually defined by protein/gene expression patterns, we have proposed applying Raman spectra to a cellular fingerprinting as an alternative for identifying the cell state, and now succeeded in predicting gene-expression of antibiotic bacteria in combination with machine learning technology. Meanwhile, SHG microscopy has been used to visualize fiber structures in living specimens, such as collagen, and microtubules as a label-free modality. By utilizing the feature that SHG senses protein structure change, we developed a new method to measure actomyosin activity in cardiac cells. The most important advantage of use of the scattering light is their non-labeling and non-invasive capability.

Symposium

2-S16-4

Deep Learning-Based System for the Research of Pluripotent Stem Cell-derived Cells

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Deep learning technology is rapidly advancing, and is now used to solve complex problems. induced pluripotent stem cells (iPSCs) can be used for several purposes such as regenerative medicine, disease modeling study and drug screening. It is inevitable to identify iPSC-derived differentiated cells in microscopy for any use. Here, we used deep learning to establish an automated method to identify endothelial cells derived from iPSCs, without the need for immunostaining or lineage tracing.

Symposium

2-S17-1

Signaling molecules hydrogen sulfide (H_2S), polysulfides (H_2S_n) and sulfite (H_2SO_3)

Hideo Kimura[1]

[1]National Institute of Neuroscience, NCNP

Since the identification of endogenous H_2S in the mammalian brain in 1989, studies of this molecule uncovered physiological roles in processes such as neuromodulation, vascular tone regulation, cytoprotection against oxidative stress. We previously demonstrated that H_2S induces Ca^{2+} influx in astrocytes by activating transient receptor potential (TRP) channels. During this study we found that H_2S_n activates TRP ankyrine 1 (TRPA1) channels much more potently than does H_2S and that 3-mercaptopyruvate sulfurtransferase (3MST) produces H_2S_2 and H_2S_3. Recently, we demonstrated that the chemical interaction of H_2S with nitric oxide (NO) generates H_2S_2 and H_2S_3 that may be a mechanism of a synergistic effect between H_2S and NO we previously showed in the regulation of vascular tone. We showed that cysteine persulfide (Cys-SSH) together with its glutathione (GSH) counterpart (GSSH), both of which have been proposed to be involved in redox homeostasis, are also produced by 3MST. We will show our recent observation that sulfite protects neurons from oxidative stress more efficiently than H_2S and H_2S_n with a distinctive mechanism.

Symposium

2-S17-2

Development and application of fluorescence probes for detecting reactive sulfur species (RSS)

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For detailed studies of the physiological functions of reactive sulfur species (RSS) such as H_2S and sulfane sulfur, we set out to develop a highly selective and easy-to-use fluorescence probe for H_2S. We designed and synthesized a novel fluorescence probe for H_2S, **HSip-1** (Hydrogen Sulfide imaging probe-1), utilizing macroazacyclic complex chemistry with copper ion (II) (***J. Am. Chem. Soc.*** 133, 18003-18005 (2011)). **HSip-1** showed the fluorescence increase (by 50 fold) within several seconds upon addition of 10 μM H_2S, whereas almost no fluorescence increment was observed upon addition of 10 mM GSH. **HSip-1** also showed high selectivity over other biothiols, ROS, and RNS. We could also visualize H_2S in HeLa cells with **HSip-1 DA** (a cell-membrane permeable derivative of **HSip-1**) upon addition of Na_2S. Moreover, we applied **HSip-1** to the detection of enzymatic activity of H_2S-producing enzymes *in vitro*. We successfully monitored the time-dependent H_2S production by 3-mercaptopyruvate sulfurtransferase (3MST) and cystathionine γ-lyase (CSE). We then applied **HSip -1** to the inhibitor high-throughput screening (HTS) of a chemical library containing 170,000 compounds from The University of Tokyo, Drug Discovery Initiative, and found selective inhibitors for 3MST and CSE (***Sci. Rep.*** 7:40227 (2017)). We also recently developed a fluorescence probe for sulfane sulfur (***Chem. Commun.***, 53, 1064-1067 (2017)).

Symposium

2-S17-3
Regulation of $Ca_v3.2$-mediated pain signals by hydrogen sulfide

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Electrophysiological, pharmacological and gene-knockdown studies have shown that hydrogen sulfide (H_2S) promotes pain or itch by enhancing $Ca_v3.2$ T-type Ca^{2+} channel activity. We thus examined the effect of $Ca_v3.2$ gene deletion on H_2S-dependent somatic or visceral pain and itch. In wild-type mice, intraplantar and intracolonic administration of Na_2S, an H_2S donor, caused somatic and colonic pain/hypersensitivity, respectively, and intradermal injection of Na_2S in the cheek evoked both pain and itch responses. These responses to Na_2S challenge disappeared in $Ca_v3.2$-KO mice. $Ca_v3.2$ deletion did not affect the partial sciatic nerve ligation (PSNL)-induced neuropathic allodynia in mice, but removed the anti-allodynic activity of T-type Ca^{2+} channel blockers. On the other hand, $Ca_v3.2$ deletion abolished endogenous H_2S-dependent bladder pain in mice with cyclophosphamide-induced cystitis, and the butyrate-induced colonic hypersensitivity in mice, models for bladder pain syndrome (BPS) and irritable bowel syndrome (IBS), respectively. Our data thus suggest that $Ca_v3.2$ plays a key role in exogenous H_2S-induced pain and itch, and in visceral pain signaling in BPS and IBS models, although unknown neuronal systems might compensate $Ca_v3.2$ deficiency in PSNL-induced neuropathy.

Symposium

2-S17-4
Availability of D-cysteine as a protectant for cerebellar neurons

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Hydrogen sulfide (H_2S) is known as a toxic gas, but has been focused as a biological mediator, which modulates signal transduction and protects cells and tissues from oxidative stress. Endogenous H_2S is mainly generated from L-cysteine, while a novel biogenesis pathway of H_2S from D-cysteine has been recently identified. In this pathway, D-amino acid oxidase (DAO) converts D-cysteine to 3-mercaptopyruvate (3MP), followed by the generation of H_2S from 3MP by 3-mercaptopyrvate sulfurtransferase. DAO is especially abundant in cerebellum among various brain regions and mediates efficient generation of H_2S from D-cysteine in the cerebellar tissues. Cerebella Purkinje cells (PCs) are characterized by the highly-branched dendrites and are important for cerebellar functions. The dendritic shrinkage and degeneration of PCs are frequently observed in patients and model mice of cerebellar ataxias. We revealed that D-cysteine enhances dendritic development of primary cultured PCs, but L-cysteine does not. This effect was inhibited by DAO inhibitors and reproduced by 3MP and a H_2S donor, suggesting that this enhancement by D-cysteine is caused by the production of H_2S. Taken together, D-cysteine would be available as a neuroprotectant against cerebellar ataxias.

Symposium

2-S18-1
Impact of P2X7 receptor on brain ischemic tolerance

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Brain ischemic tolerance is an endogenous neuroprotective mechanism, whereby an experience of non-lethal ischemic episode (preconditioning; PC) produces resilience to subsequent lethal ischemia. We previously showed that PC caused activation of astrocytes and a subsequent upregulation of P2X7 receptors, activation of which induced ischemic tolerance via upregulation of HIF-1α in astrocytes. P2X7 receptor requires relatively higher extracellular ATP concentrations (ATPo) for its activation. However, the PC-evoked increase in ATPo was not enough to activate P2X7 receptor. Here, we show that astrocytes have a unique mechanism of P2X7 receptor activation with lower ATPo, thereby leading to ischemic tolerance. It has been reported that NAD at lower ATPo could induce prolonged activation of P2X7 receptor via ART2-catalyzed ADP-ribosylation. Thus, we tested effect of NAD on astrocytic P2X7 receptors, and found that NAD increased HIF-1α in WT astrocytes but not in P2X7 receptor-deficient astrocytes *in vitro*. We also found that ART2 is selectively expressed and upregulated by PC in astrocytes. Taken together, our findings suggest that astrocytes could activate P2X7 receptors by their unique mechanisms, i.e., NAD/ART2/P2X7 signal pathways and induce ischemic tolerance.

Symposium

2-S18-2

Regulation of VNUT-mediated vesicular ATP release in nervous system and immune system

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During the purinergic chemical transmission, neurons, neuroendocrine cells, glial cells, immune cells and other types of cells secrete ATP, and communicate with each other through purinoceptors on the plasma membrane. In spite of well-understood features on the signaling cascade after stimulation of the purinoreceptors, the mechanism of how ATP is stored and released from the purinergic cells is far less characterized. In this study, we focus on the mechanism on vesicular secretion through vesicular nucleotide transporter (VNUT). VNUT transports nucleotides such as ATP and ADP and plays an essential role in the vesicular storage and secretion of ATP. Recently, we identified the clinically available VNUT inhibitor clodronate, which impaired vesicular ATP release from neurons, microglia, and neutrophils. Clodronate also impaired neutrophil migration and attenuated neuropathic and inflammatory pain *in vivo*. Although more extensive works will be necessary, these studies show that VNUT-specific inhibitor is able to control vesicular ATP release *in vivo* and that VNUT regulates purinergic chemical transmission.

Symposium

2-S18-3
Extracellular nucleotides induce glutamatergic subtype neurons

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Extracellular nucleotides can control proliferation of neural stem cells (NSCs). However, their effects on selection of neuronal subtypes have not been elucidated. Glutamatergic neurons are the most abundant subtype in the mammalian brain. Production of this neuronal subtype can be observed not only in the development of the forebrain, but also through life in the hippocampus. In the development, glutamatergic neurons are produced from neuroepithelial cells at the dorsal side of the anterior neural tube. In the adult hippocampus, stem cells located at the sub-granular zone can produce glutamatergic granule neurons through life. We found that the expression of the nucleotide receptor P2Y4 was transiently augmented in the course of neuronal differentiation of mouse ES cells. Interestingly, a subpopulation of type 2 NSCs of the adult mouse hippocampus also expressed P2Y4. The activation of P2Y4 in those cells increased proportion of glutamatergic subtype in their descendant neurons. Our results provide evidence that differentiating NSCs pass through a stage in which nucleotides can affect subtype marker expression of their descendant neurons.

Symposium

2-S18-4
Glial purinergic signals as a target for antidepressants

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Although psychotropic drugs act on neurons and glial cells, how glia respond, and whether glial responses are involved in therapeutic effects are poorly understood. Here, we show that fluoxetine (FLX), an anti-depressant, mediates its anti-depressive effect by increasing the gliotransmission of ATP. FLX increased ATP exocytosis via vesicular nucleotide transporter (VNUT). FLX-induced anti-depressive behavior was decreased in astrocyte-selective VNUT-knockout mice or when VNUT was deleted in mice, but it was increased when astrocyte-selective VNUT was overexpressed in mice. This suggests that VNUT-dependent astrocytic ATP exocytosis has a critical role in the therapeutic effect of FLX. Released ATP and its metabolite adenosine act on $P2Y_{11}$and adenosine A2b receptors expressed by astrocytes, causing an increase in brain-derived neurotrophic factor in astrocytes. These findings suggest that in addition to neurons, FLX acts on astrocytes and mediates its therapeutic effects by increasing ATP gliotransmission.

Symposium

2-S19-1

Development of cancer-specific monoclonal antibodies against glycoproteins

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Many strategies have been tried to produce monoclonal antibodies (mAbs); however, there have been several problems about focusing on molecular targets and screening methods. For instance, the high tumor/normal ratio of antigen expression using DNA microarray has been thought to be important when we determine the molecular targets for antibody-drug. Although many antigens are expressed highly in tumors, those antigens have been removed from the candidates of antibody-drug targets because they were also expressed in normal tissues. We recently established a novel technology to produce a cancer-specific monoclonal antibody (CasMab). The post-translational difference such as glycans can be utilized to produce the CasMab, although the protein possesses the same amino acid sequence in both cancer and normal cells. We have already produced CasMabs against several glycoproteins such as podoplanin, which is expressed in both cancer and normal cells. Those CasMabs possess antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) in vitro and anti-tumor effect in xenograft models in vivo. In conclusion, the CasMab technology is the platform to develop cancer-specific mAbs, which could attack only cancer cells without side effects.

Symposium

2-S19-2

Multiple functions of plasma histidine-rich glycoprotein and its clinical application as a biomaterial

Hidenori Wake[1], Masahiro Nishibori[1]

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Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. Neutrophil-associated inflammation or microthrombus formation (Immunothrombosis) in the lung resulted in acute respiratory distress syndrome, the most important cause of death in septic multiple organ failure. Histidine-rich glycoprotein (HRG) is a 75 kDa glycoprotein mainly produced by liver. HRG is known as the plasma factor to regulate coagulation/fibrinolysis, immune response and angiogenesis. Our recent studies revealed that plasma HRG levels significantly decreased in cecal ligation puncture (CLP) septic mice model and administration of HRG dramatically improved the survival rate of CLP mice associated with the inhibition of immunothrombosis and neutrophil extracellular trap formation in pulmonary vasculatures by keeping neutrophils quiescent morphologically and functionally, and the protection of vascular endothelial cells and inhibition of erythrocyte aggregation. Thus, HRG-supplementary therapy may provide a novel strategy for the treatment of septic patients.

Symposium

2-S19-3
Radiolabeled proteins for the development of biopharmaceuticals

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Positron emission tomography (PET) is a powerful tool for drug discovery because it would enable the evaluation of target engagement (proof-of-concept), pharmacokinetics, and therapeutic efficacy of drug candidates in humans using PET radiopharmaceuticals. Recently, biopharmaceuticals are attracting increasing interest because they possess not only high binding affinity and specificity but also creates unique actions to attack pathologic lesions, requiring the radiolabeling methods of proteins to evaluate their pharmacokinetics and efficacy. In this presentation, we review the radiolabeling methods for the biopharmaceutical itself using long-half lived PET radionuclides such as Cu-64 and Zr-89, which are suitable for the evaluation of their pharmacokinetics. Since Cu-64 and Zr-89 showed relatively high radiation, shorter half-lived radionuclides such as F-18 is better from the point view of radiation. Here, we show our recent developed approach for the production of F-18 labeled proteins, which are suitable for the evaluation of their therapeutic efficacy and patient selection with minimum radiation.

Symposium

2-S20-1

Possible contribution of PACAP-evoked spinal astrocyte-neuron lactate shuttle to the chronic pain development

Takashi Kurihara[1], Yuki Kambe[1], Masafumi Yokai[1], Ayaka Shimodaira[2], Ichiro Takasaki[2], Atsuro Miyata[1]

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Previously, we showed that spinal pituitary adenylate cyclase-activating polypeptide (PACAP)/PAC1 receptor signaling triggers long-lasting pain-like behaviors through astroglial activation. Since astrocyte-neuron lactate shuttle (ANLS) could be essential for long-term synaptic plasticity, we aimed to elucidate a possible involvement of spinal ANLS in the development of the PACAP-evoked pain-like behaviors. A single intrathecal administration of PACAP induced short-term spontaneous aversive behaviors, followed by long-lasting mechanical allodynia in mice. These behaviors were inhibited by DAB, an inhibitor of glycogenolysis, and this inhibition was reversed by simultaneous L-lactate application. In the cultured spinal astrocytes, the PACAP-evoked glycogenolysis and lactate secretion were inhibited by a protein kinase C (PKC) inhibitor, and the PKC inhibitor attenuated the PACAP-induced pain-like behaviors. Moreover, an inhibitor for the monocarboxylate transporters blocked the lactate secretion from the spinal astrocytes and inhibited the PACAP-evoked pain-like behaviors. In this symposium, we will further discuss possible involvement of the spinal PACAP-ANLS signaling in an experimental model of neuropathic pain.

Symposium

2-S20-2

Involvement of astrocyte-neuron-lactate shuttle in neuropathic pain

Masahiro Ohsawa[1], Keisuke Miyamoto[1], Kei-Ichiro Ishikura[1], Rina Ueda[1], Daisuke Uta[2], Kazuhiko Kume[1]

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Astrocytes play a key role in the maintenance of synaptic transmission by producing L-lactate via the astrocyte-neuron lactate shuttle (ANLS). Astrocyte activation in the spinal cord is involved in the expression of neuropathic pain. These reactive astrocytes are suggested to play an important role in the maintenance of neuropathic pain. We investigated the role of the ANLS in the spinal cord on hyperalgesia in neuropathic pain in mice. We also investigated the cellular mechanisms of spinal L-lactate-induced mechanical hyperalgesia. We revealed that the selective activation of spinal dorsal horn astrocytes causes mechanical hyperalgesia through the excessive L-lactate supply to neurons via monocarboxylate transporters (MCTs). We also found that L-lactate transported into neurons may produce mechanical hyperalgesia. In addition, application of L-lactate enhanced the excitatory neurotransmission evoked by mechanical stimulation in the dorsal horn of spinal cord. Moreover, intrathecal treatment with L-lactate also activates protein kinase A (PKA) signaling. These results suggest that the enhanced ANLS sensitizes the nociceptive transmission in the spinal cord through the activation of PKA signaling.

Symposium

2-S20-3

Specific contribution of monocarboxylate transpoter-dependent energetic supply in the brain network underlying nociception-emotion link

Fusao Kato[1], Masashi Nagase[2], Ryota Eguchi[3]

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The excitatory transmission from the solitary tract (TS) primary afferents to the second-order neurons in the nucleus of the solitary tract (NTS) depends largely on the energetic supply through monocarboxylate transporters (MCTs) (Nagase et al., 2014). We examined whether this large dependency of the excitatory transmission on lactate supply observed in the TS-NTS synapse is commonly shared by brain circuits underlying pain as "sensory and emotional experience". The lateral parabrachial nucleus (LPB) is the site where the nociceptive information arising from the spinal dorsal horn and trigeminal nerve converges and functions as a relay of these signals to the pain-associated networks. The central amygdala (CeA) is the most important target of the ascending LPB projections, which undergoes robust synaptic potentiation in various pain models (Kato et al., 2018). In brain slices prepared from rats and mice, the excitatory synaptic transmission from the LPB to the CeA was significantly attenuated by inhibition of MCTs with 4-hydroxycinnamic acid (4-CIN) without significant changes in paired-pulse ratio. This effect was similarly observed in setups where LPB fibers were stimulated electrically and optogenetically. Interestingly, in the current clamp recordings, blockade of MCTs resulted in postsynaptic depolarization in the CeA neurons, unlike in the pyramidal neurons in the lateral amygdala, hippocampal CA1 and Purkinje neurons in the cerebellum, which were hyperpolarized by activation of K_{ATP} channels. As the excitability of CeA neurons is a crucial determinant of the emotional/aversive aspects of pain, the pharmacological regulation of the lactate transport in the CeA would be a candidate as a target for resetting aberrantly augmented nociception-emotion link and malfunctioning descending regulation.

Symposium

2-S20-4

Involvement of human monocarboxylate transporters (MCTs) in the uptake of L-lactate in human astrocytes and identification of a selective inhibitor of MCTs

Masaki Kobayashi[1]

[1]Dept. Pharm., Hokkaido Univ. Hosp.

Astrocyte-neuron lactate shuttle (ANLS) signaling contributes to learning or memory and induces long-lasting nociceptive behavior. Monocarboxylate transporters (MCTs) have been reported to transport L-lactate. While MCT1, MCT2, and MCT4 are expressed in the rodent brain, it remains unclear which MCT isoform is functionally expressed by human astrocytes. Furthermore, there have been only a few reports on MCT1- or MCT4-selective inhibitors. First, we established the contribution of each MCT isoform to L-lactate transport in human astrocytes. The cellular uptake of L-lactate was found to be pH- and concentration-dependent with a Km value of 0.64 mM for L-lactate uptake. This Km value was similar to that previously established for MCT1-mediated L-lactate uptake. Next, we tried to identify a selective MCT1 or MCT4 inhibitor. While inhibitory effects were observed for MCT1 with 5-oxoproline, no inhibitory effect was observed for MCT4. Bindarit was demonstrated to exhibit selective inhibitory effect against MCT4, but not against MCT1. These findings provide novel insights into the mechanism of L-lactate transport by astrocytes, and contribute to developing novel targets for ANLS.

Symposium

3-S21-1
Novel regulatory mechanism of gene expression by nitric oxide

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Nitric oxide (NO) is a highly diffusible molecule generated from a family of NO syntheses (NOSs) that convert L-arginine to L-citrulline using oxygen and NADPH. Under physiological conditions, NO is produced at low levels in response to endogenous stimuli such as acetylcholine and acts as critical messenger of intracellular signaling pathways. NO also has a variety of physiological functions such as neurotransmission, inflammatory reaction, vasodilation, and stimulates both angiogenesis and cell proliferation.

Protein *S*-nitrosylation is an NO-dependent reversible post-translational modification of cysteine that regulates both protein structure and function in bacteria, plants, and mammals. Over the past couple of decades, advanced proteomic approaches have led to the identification of more than a thousand *S*-nitrosylated (SNO-) proteins with diverse cellular functions. So far, we demonstrated that PDI, PTEN, and HDAC6 are targets of NO and regulate the enzymatic activity via *S*-nitrosylation.

Recently, we found a novel substrate of NO that is involved in the regulation of gene expression. In addition, we attempted to develop a specific inhibitor of *S*-nitrosylation of this substrate by the hierarchical virtual screening approaches. We will discuss those findings in this session.

Symposium

3-S21-2

Signaling by hydrogen polysulfides (H_2S_n) produced by the chemical interaction between hydrogen sulfide (H_2S) and nitric oxide (NO)

Hideo Kimura[1]

[1]National Institute of Neuroscience, NCNP

H_2S is produced by enzymes and has various physiological roles including neuromodulation, vascular tone regulation, cytoprotection against oxidative stress. We previously demonstrated that H_2S relaxes vascular smooth muscle in synergy with NO. In the process of the study about the effect of H_2S on transient receptor potential (TRP) channels, we found that H_2S_n activates TRP ankyrine 1 (TRPA1) channels much more potently than does H_2S and that 3-mercaptopyruvate sulfurtransferase (3MST) produces H_2S_2 and H_2S_3. The chemical interaction of H_2S with nitric oxide (NO) has been reported to generate several products including nitroxyl (HNO), nitrosopersulfide (HSSNO) and H_2S_n. The effect of H_2S_n on TRPA1 channels is suppressed by reduction and by cyanide, while that of HNO is resistant to reduction and that of HSSNO to cyanide. Based on these observations we concluded that H_2S_n are chemical entities generated by the interaction of H_2S with NO. I will focus on the production of H_2S_n and a potential mechanism of the synergistic effect of H_2S and NO.

Symposium

3-S21-3

Anti-metabolic effect of dietary NOx and lung-protective effect of myelocytic NOSs

Masato Tsutsui[1]

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In this symposium, we introduce our recent studies regarding new actions of diet-derived nitric oxide (NO) and bone marrow-derived NO.

A new pathway in which NO is synthesized from NO metabolites; nitrite and nitrate (NOx), is recently discovered. However, whether dietary NOx deficiency causes diseases remains to be clarified. We demonstrated that long-term low NOx diet caused metabolic syndrome, vascular dysfunction, and cardiovascular death in mice. These results indicate that dietary NOx plays a pivotal role in the prevention of those disorders (*Diabetologia* 2017).

NO, synthesized by NOSs (nNOS, iNOS, and eNOS), plays a role in the development of pulmonary hypertension. However, the role of NO/NOSs in bone marrow cells in pulmonary hypertension remains elusive. We showed that transplantation of n/i/eNOSs-deficient bone marrow significantly aggravated hypoxia-induced pulmonary hypertension in wild-type mice, and transplantation of wild-type bone marrow significantly ameliorated hypoxia-induced pulmonary hypertension in n/i/eNOSs-deficient mice. These results show that myelocytic n/i/eNOSs play a crucial protective role in the pathogenesis of pulmonary hypertension (*Am J Respir Crit Care Med* 2018).

Symposium

3-S21-4

The Role of NO in the Progression and Regression of Atherosclerosis via Endothelial Senescence

Toshio Hayashi[1], Morihiko Maeda[1], Tomoe Tsuboi[2]

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Nitric oxide(NO) bioavailability is limited in senescence. We studied it in human umbilical venous endothelial cells(HUVECs). NO donor and transfection with endothelial NO synthase(eNOS) into HUVECs each decreased SA-b-gal positive cells and increased telomerase activity. 17b-estradiol decreased SA-b-gal-positive cells and caused cell proliferation. L-arginine(L-Arg) or L-citrulline(L-Cit) partially inhibited, and combination of L-arg and L-cit(LALC) strongly prevented, high glucose-induced senescence. Following 3-day stimulation of HUVECs under high-glucose with L-Arg or L-Cit or LALC, endothelial senescence and function were evaluated. These amino acids were also administered to dyslipidemic type 2 diabetic(ZDFM) rats fed a high-cholesterol diet for 4 weeks. L-Cit and LALC retarded HG-induced endothelial senescence, and restored telomerase activity. p22-phox was not altered, but L-Cit decreased ROS. Under HG, L-Cit and LALC increased NO. and eNOS and phosphorylated eNOS were decreased. In ZDFM rats, SA-b-gal on the aortic surface was reduced by L-Cit and LALC. LALC for 4 weeks increased plasma NO. Thus, L-Cit and LCLA inhibited HG-induced endothelial senescence and NO-cGMP pathway. The delay in endothelial senescence through NO and eNOS may have clinical utility in the treatment of atherosclerosis in elderly.

Symposium

3-S22-1

Cardio-oncology -Elucidation of the mechanism of cardiac dysfunction caused by cancer therapy and cancer cachexia-

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Cancer patients nowadays can choose a wide variety of cancer therapies based on new anti-cancer drugs and state-of-the art operational technology. Also, cancer has been recognized as curable diseases with increased cancer survivors. On the other hand, cancer survivors who have various problems during and after treatment of cancer therapy are increasing. Recently, as an increase in the number of cancer survivors, emphasis has been placed on the importance of development of survivors' QOL such as cardiovascular disorders occurred in cancer survivors. Indeed, certain anticancer drugs and molecular targeted therapies induce cardiotoxicity, which limit the widespread implementation of cancer treatment and significant decrease of QOL in cancer patients. In addition, cardiac dysfunction induced by cancer cachexia has also been reported. In view of these backgrounds, it is necessary to clarify the dynamics of cardiovascular system of cancer survivors and patients with cancer cachexia, to prevent cardiac dysfunction accompanying with cancer therapy and cachexia. Quite recently, interdisciplinary research area namely Cardio-oncology has been established to try to solve these issues. In the present study, we will present anticancer drug-induced cardiotoxicity and cardiac dysfunction occurring in cancer cachexia, and discuss our research approaches and data to understand such pathophysiology and possible prevention and therapies.

Symposium

3-S22-2

Human iPS cell technology as non-clinical tools for predicting cardio-oncology therapy side effects

Yasunari Kanda[1]

[1]Div Pharmacol, NIHS

As cancer patients live longer, it is important to recognize cardiotoxicity that induce electrophysiological or structural changes by many oncology drugs, such as anthracyclines and tyrosine kinase inhibitors. Thus, understanding of cardio-oncology (also known as onco-cardiology) is more critical for the effective care of cancer patients. Huma induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have been widely used to evaluate cardiotoxicity as highly physiologically relevant human cells. We all-japan consortium have proposed a new proarrhythmia risk assessment method using hiPSC-CMs by our large-scale validation study. In addition to proarrhythmia risk, it is necessary to detect other type of side effects, such as chronic effects and impaired contractility from the viewpoint of cardio-oncology. To investigate how to use hiPSC-CMs for oncology drugs, we have attempted to make standardized protocols for long-term effects of drugs using motion vector system. In the symposium, I would like to share our recent data and discuss future perspectives for the usage of hiPSC-CMs in the cardio-oncology field.

Symposium

3-S22-3

The role of DNA methylation in anticancer drug-induced DNA damage repair system in cardiomyocytes.

Hiroshi Hosoda[1]

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Genomic DNA, which contains all of the genetic information, is damaged by a variety of endogenous and environmental factors such as genotoxic chemicals, ionizing radiation and UV light. Consequently, the DNA repair process is constantly active as it responds to damage in the DNA structure. Not only cardiotoxicity of anticancer drug treatment but also ischemic heart disease and heart failure associated with overloaded pressure interfere with DNA damage response and DNA repair regulation in cardiomyocytes. DNA methylation, catalyzed by the DNMTs, plays an important role in maintaining genome stability, but the molecular mechanism is not clear. In this study, we examine and outline the links between DNA methylation and the DNA damage repair systems and discuss the possible mechanisms of how they are orchestrated, with a focus on cardiotoxicity of anticancer drugs.

Symposium

3-S22-4

Cardio-Oncology: Challenges and Opportunities in the New Interdisciplinary Research

Kazuhiro Sase[1,2]

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Advances in cancer treatment have led to dramatic increase in cancer survivors.
Although cardiotoxicity resulting from anthracyclines and radiation therapies has been known for decades, the emergence of novel cancer treatment-related cardiovascular diseases (CTRCD) have been recognized to be associated with molecularly targeted therapies as well as immune checkpoint inhibitors.
Cardio-Oncology is a new interdisciplinary research opportunity at the intersection of cardiovascular disease and cancer.
Research priorities need to be identified to diagnose, treat, and prevent the previously unknown CTRCD(s), including (1) myocardial dysfunction and heart failure, (2) coronary artery disease, (3) valvular disease, (4) arrhythmias and QT-prolongation, (5) arterial hypertension, (6) thromboembolic disease and others.
For example, understanding fundamental mechanisms underlying CTRCD is essential to the development of new methods to manage these toxicities. The application of more suitable disease models and more effective methods for toxicity screening will serve to advance our understanding of CTRCD. Animal models have been successfully employed to predict potential problems in some cases, but more highly predictive models are also needed. Biobanks and other specimens with patient registries would facilitate the validation of biomarkers, genome analysis, and imaging methods.

Symposium

3-S23-1
ILC2 induce innate IgE secretion by B1 cells via IL-4 production

Kazuyo Moro[1,2,3]

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Group 2 innate lymphoid cells (ILC2s), a new type of innate lymphocyte that we originally reported as natural helper cells in 2010, are known to regulate type 2 immune responses in an antigen-independent manner. In contrast to Th2 cells, ILC2s lack rearranged antigen receptors and are directly stimulated by epithelial cell-derived cytokines such as IL-33 and IL-25. Activated ILC2s are capable of producing a variety of cytokines, chemokines, and peptides including IL-5, IL-6, IL-9, IL -13, GM-CSF, amphiregulin, eotaxin, and methionine-enkephalin. ILC2s play a vital role in protection against parasite infections and induction of eosinophilic inflammation, which is involved in asthma, atopic dermatitis, eosinophilic esophagitis, allergic rhinitis, and chronic rhinosinusitis with nasal polyps. ILC2s are now considered to be associated with a wide range of diseases including allergic diseases, infection, obesity, cancer, and fibrosis.

IL-4, a key type 2 cytokine, is involved in multiple immune reactions including Th2 cell development, IgG1 and IgE production by B cells and M2 macrophage differentiation. While Th2 cells produce IL-4 together with IL-5 and IL-13 under antigen-induced TCR stimulation, ILC2s fail to produce IL-4 under IL-33 stimulation, which induces a large amount of IL-5 and IL-13. Based on this fact, ILC2s are not thought to contribute to IL-4-mediated immune responses, even though they express high levels of the IL-4 gene.

Recently, we identified the physiological condition that induces IL-4 production from ILC2. The mechanisms for IL-4 production from ILC2 were more intricate than that in Th2 cells and differ widely from those in IL-5 and IL-13 production. Furthermore, IL-4 from ILC2s elevates polyclonal IgE levels in steady state, helminth infection and allergy, and supports survival and expansion of $Fc\varepsilon R^{+}$ cells such as basophils and mast cells. These findings provide evidence for factors involved in susceptibility to allergic diseases, which is still not understood, but is an important issue in the treatment or prevention of allergic disorders.

Symposium

3-S23-2

The role of inflammatory cytokines in the pathogenesis of pulmonary arterial hypertension

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Pulmonary arterial hypertension (PAH) is a serious disease characterized by arteriopathy in the small to medium-sized distal pulmonary arteries, that is associated with arterial muscularization, concentric intimal thickening, and the formation of plexiform lesions. Inflammation and autoimmunity are currently thought of as critical factors to the pathogenesis of PAH. Interleukin-6 (IL-6), a multifunctional pro-inflammatory cytokine, is elevated in the serum of pulmonary arterial hypertension (PAH) patients and can predict the survival of idiopathic (I)PAH patients. Previous animal experiments and clinical human studies indicate that IL-6 is important in the pathogenesis of PAH. We recently found that IL-6/IL-21 signaling axis plays a critical role in the pathogenesis of PAH (*PNAS*. 112(20): E2677, 2015). First, we found that IL-6 blockade by the monoclonal anti-IL-6 receptor antibody, MR16-1, ameliorated hypoxia-induced pulmonary hypertension (HPH) and prevented the hypoxia-induced accumulation of Th17 cells and M2 macrophages in the lungs. Furthermore, the hypoxia-induced upregulation of IL-17 and IL-21, which are primarily produced by Th17 cells, was also ameliorated by IL-6 blockade in mice. Whereas IL-17 blockade with an anti-IL -17 neutralizing antibody had no effect on HPH, IL-21 receptor-deficient mice were resistant to HPH and exhibited no significant accumulation of M2 macrophages in the lungs. Consistently, IL-21 indeed promoted the polarization of primary alveolar macrophages toward the M2 phenotype. Moreover, significantly enhanced expressions of IL-21 and M2 macrophage markers were detected in the lungs of IPAH patients who underwent lung transplantation. We are currently examining the effect of IL-21-blockade on the pathogenesis of severe PAH rat model (namely Sugen5416(Su) /hypoxia (Hx) PAH model). IL-21 receptor deletion significantly ameliorated the pathologies of Su/Hx PAH in rats. Taken together, these findings indicate that IL-21blockade might be a promising therapeutic option for refractory PAH. We would like to validate the therapeutic effect of IL-21 blockade for refractory patients with PAH in the near future.

Symposium

3-S23-3

Tyrosine kinase FYN inhibition mediates the therapeutic effects of Eicosapentaenoic acid on pulmonary hypertension

Hai Lin Kurahara[1], Keizo Hiraishi[1], Aya Yamamura[2], Ying Zhang[3], Narumi Shioi[4], Ryuji Inoue[1]

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Background and Purpose: Pulmonary arterial hypertension (PAH) is a multifactorial disease characterized by pulmonary arterial remodeling in which the Src family non-receptor tyrosine kinases including Fyn play non-trivial roles. In this study, we explored the therapeutic potential of eicosapentaenoic acid (EPA) and its metabolite resolvin E1 (RvE1) for PAH through inhibition of Fyn *in vitro*and *in vivo*.

Method: Cardiodynamic parameters of rat hearts were measured by the echocardiography. Contractile responses of isolated pulmonary arteries were examined by the isometric tension measurement. Proliferation of human pulmonary artery smooth muscle cells (HPASMCs) derived from PAH patients were evaluated by the MTT assay. Stress fiber formation and STAT3 phosphorylation in HPAECs and HPASMCs were examined by immunohistochemical and western blot analyses, respectively.

Results : Administration of EPA to MCT-treated rats significantly improved the pathological changes characteristic for PAH, i.e. pulmonary arterial thickening, right ventricle dysfunction and cardiovascular fibrosis. Pulmonary arteries from MCT-treated rats showed exaggerated contractile responses compared with those from vehicle-treated rats, which were greatly normalized by EPA treatment. Administration of EPA or RvE1 decelerated the enhanced proliferation of PAH patient-derived PASMCs. Immunocytochemical and western blot analyses showed that a dominant negative form of Fyn prevented TGF-β2-induced stress fiber formation and IL-6-induced STAT3 phosphorylation. EPA and RvE1 suppressed Src family activity by modulating it's autophosphorylation level.

Summary: EPA significantly improved PAH-associated pathophysiology and cardiac dysfunction, which is likely mediated at least in part via Fyn inhibition. These results also point to the therapeutic significance of targeting this molecule in PAH treatment.

Symposium

3-S23-4
The role of TRPM7 in fibrosis associated heart diseases

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Cardiac fibrosis is a hallmark of various heart diseases including hypertrophy, heart failure, and arrhythmia. Cardiac fibroblasts play an important role in fibrogenesis because they differentiate to myofibroblasts under various pathological conditions. Thus, targeting cardiac fibroblast differentiation to attenuate fibrosis represents a new therapeutic strategy for fibrosis associated heart diseases. We have previously used fibroblasts from atrial fibrillation (AF) patients and demonstrated that the Transient Receptor Potential Melastatin 7 (TRPM7) plays an essential role in fibroblast differentiation to myofibroblasts. Here we propose that TRPM7 plays a key role in fibrosis-associated arrhythmia. We used transverse aortic restriction (TAC) induced hypertrophy/heart failure mouse model to generate fibrosis in the hearts. We found that deletion of *Trpm7*(TRPM7-KO) significantly increased survival rate after TAC, and improved heart performance. Moreover, TRPM7 deletion drastically reduced fibrosis in both atria and ventricles. The reduced fibrosis in TRPM7-KO-TAC mice significantly decreased the vulnerability of AF and the duration of induced AF. Thus, TRPM7 plays an important role in fibrosis associated AF, and may serve as a therapeutic target for fibrosis associated arrhythmia.

Symposium

3-S24-1
Functional identification of neurons regulate sleep and wakefulness

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Sleep/wakefulness state change is regulated by neurons, however, its regulatory mechanism is still unclear. Here we found that GABAergic neurons in the ventral tegmental area (VTA) have an important role in the regulation of sleep/wakefulness. Adeno-associate virus (AAV) vectors were injected into VTA of glutamic acid decarboxylase (GAD)-Cre mice in which GABAergic neurons are exclusively express Cre. To manipulate GABAergic neurons in the VTA, channelrhodopsin2 (ChR2), anion-channelrhodopsin2 (ACR2) or hM3Dq was expressed by AAV. Chemogenetical activation of these neurons significantly increased time in NREM sleep. To reveal neural mechanism, slice patch clamp was performed. AAV was injected to VTA to express ChR2 in the GABAergic neurons in the VTA. Orexin neurons expressing fluorescent protein were identified and recorded. Then, GABAergic nerve terminals from VTA were stimulated by blue light. Blue light significantly inhibited activity of orexin neurons. On the other hand, optogenetical inhibition of these neurons using ACR2 immediately induced wakefulness. To evaluate physiological importance of this response, these neurons were inhibited during recovery sleep after sleep deprivation for 4 hr. Inhibition of GABAergic neurons induced wakefulness even in the very sleepy condition. These results suggest that activity of GABAergic neurons in the VTA is critical to change sleep/wakefulness state especially in the regulation of NREM sleep and wakefulness.

Symposium

3-S24-2
Stress-induced responses in the central-peripheral associations

Takuya Sasaki[1]

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Peripheral organ functions such as cardiovascular and respiratory activity are controlled by the nervous system. Though most early studies have mainly focused on physiological events within a single organ, it remains largely unknown how the central nervous system and peripheral organs interact with each other. To address this issue, we hereby developed a recording method that comprehensively monitors electrical biosignals representing cardiac rhythm, breathing rhythm, awake/sleep-related muscle contraction, and collective neuronal activity of multiple brain regions. Using this novel technique, we examined physiological changes in central-peripheral activity in rats that were subject to social defeat stress. Rats were classified into stress-susceptible and stress-resilient groups, based on cardiac and respiratory signals. Multi-dimensional discriminant analysis revealed that certain activity patterns of cortical oscillations could predict future animal's susceptibility against stress. Furthermore, stress-susceptible animals exhibited decreases in activity levels in multiple brain regions, including the hippocampus, the somatosensory cortex, and the thalamus. Such dynamic changes in cortical activity is a possible mechanism to cause abnormal activity of the peripheral organs in response to mental stress episodes.

Symposium

3-S24-3

Neural circuitry system of the response selection and switching flexibility

Shigeki Kato[1], Kazuto Kobayashi[1]

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Although previous studies have indicated that various brain areas involve in the acquisition of associative learning, it still remains unclear how the neural circuitry system regulates the learning processes. In order to clarify these mechanisms, we have developed retrograde vectors which are applicable as an approach for the study of neural circuits based on brain functions by combining with cell targeting, opto/chemogenetics in selective neural pathways. By using these methods, we addressed the roles of the pathways originating from the parafascicular nucleus (PF) and central lateral nucleus (CL) in the intralaminar thalamic nuclei to the striatum in mice. Interestingly, these two pathways control both the acquisition and performance of visual associated discrimination. In addition, the elimination of the CL-derived thalamostriatal neurons impaired the behavioral switching flexibility through the reversal and the set shifting tasks. Our data suggest that the PF and CL thalamostriatal systems are involved in cognitive function of basal ganglia circuitry, and that these two circuits possess distinct roles in the control of behavioral selection and flexibility.

Symposium

3-S24-4

Pharmacological multiple analysis of specific-subpopulation by applying of cell-labeling technique

Naoko Kuzumaki[1,2], Moe Watanabe[1,3], Minoru Narita[1,2]

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Our understanding of brain function under the drug treatment and pathology has been promoted by genetically encoded tools for labeling *neuronal subpopulation* and manipulating neuronal function. Recent studies have indicated that ventral tegmental area (VTA) dopamine (DA) neurons are heterogeneous in their properties. In the present study, we demonstrated that morphine (MRP)-responsive neurons were isolated from the VTA of c-Fos-EGFP/Rpl10a transgenic mice expressing an EGFP fused to *Rpl10a*, under the control of the *c-fos* promoter and purified using MACS and FACS. These experiences showed that systemic administration of MRP activated a subset of VTA neurons, including TH-positive DA neurons. Furthermore, using c-Fos-eNpHR mice, optical suppression of MRP-responsive VTA neurons significantly inhibited analgesic responses induced by systemic administration of MRP. These results suggest that the activation of MRP-responsive mesolimbic DA neurons partly modulates MRP-induced analgesia. The present findings provide evidence that labeling *neuronal subpopulation* and manipulating neuronal function using genetic engineering technology is very powerful tool for detecting functional neural circuits under drug treatment and disease conditions.

Symposium

3-S25-1

Effect of alpha1 adrenoceptor antagonist on lower urinary tract symptoms as a vasodilative agent

Shogo Shimizu[1], Takahiro Shimizu[1], Youichirou Higashi[1], Motoaki Saito[1]

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Lower urinary tract symptoms (LUTS) are categorized as storage, voiding, and post-micturition symptoms of the lower urinary tract. Recent clinical evidence have shown that hypertension and atherosclerosis resulting in disturbed blood flow are linked to the development of LUTS. Spontaneously hypertensive rat (SHR) is genetically hypertensive rat and shows various urinary disorder model such as detrusor overactivity/overactive bladder and benign prostatic hyperplasia (BPH) with a decrease in pelvic blood flow. Alpha1 adrenoceptor antagonists are widely used as the treatment of BPH/LUTS. Moreover, there is increasing evidence that alpha1 adrenoceptor antagonists improve the voiding symptoms as well as storage symptoms in BPH/LUTS patients. We reported that chronic daily treatment with silodosin (a selective alpha1A adrenoceptor antagonist) ameliorated the decreased bladder blood flow and urodynamic parameter in SHR. Moreover, chronic treatment with silodosin improved the prostatic morphological changes and decreased prostatic blood flow in SHR. These data suggest that silodosin might improve the LUTS via a recovery of pelvic blood flow.

Symposium

3-S25-2
Afferent Nerve Activity in Relation to Bladder Sensation

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Bladder afferent nerves are composed by myelinated Aδ- and unmyelinated C-fibers. During the storage phase of urine, distention of the bladder has long been considered to evoke afferent activity via Aδ-fibers connected in series with the smooth muscle fibers. In contrast, a previous study in cats revealed that more than 90% of C-fibers do not respond to normal bladder distension, being so called "silent" fibers. However, at least in rats, C-fibers can respond to normal bladder distension like Aδ-fibers, although they may also fulfill a potentially different role in the bladder sensory function in response to abnormal stimuli.

The symptoms of overactive bladder (OAB) or interstitial cystitis (IC) are believed to be commonly related to the sensory (afferent) function. In our laboratory, a direct measuring technique of mechanosensitive single-unit afferent activities of the primary bladder nerves in the rat has been established, and we have investigated the direct effects of drugs (anticholinergics, β3-adrenoceptor agonists, α1-adrenoceptor antagonists, PDE type5 inhibitors, etc.) on the bladder afferent function.

In this symposium, we will show some of our results and propose a possible additional action on sensory pathway of drugs as therapeutic agents for OAB or IC.

Symposium

3-S25-3

Effect of tadalafil on chronic pelvic pain in two types of rat nonbacterial prostatitis model□

Hiroshi Yamaguchi[1], Maki Kurita[1], Ryohei Yoshinaga[1], Yasunori Asao[1], Michiko Oka[1]

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Tadalafil has recently been reported to improve the International Prostatic Symptom Score and total National Institutes of Health Chronic Prostatitis Symptom Index score of patients with benign prostatic hyperplasia with chronic pelvic pain syndrome (Nishino et al., Hinyokika Kiyo 2017 **63**:101-105). We therefore investigated the effect of tadalafil on chronic pelvic pain, which is a hallmark of chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS), and the inflammatory changes in two types of rat CP/CPPS model: experimental autoimmune prostatitis (EAP) and prostatitis induced by 17β-estradiol treatment combined with castration (hormone/castration-induced prostatitis; HCP) (Okamoto et al., The Prostate 2018 **78**:707-713; Kurita et al., The Prostate 2018 **78**:1157-1165; Yamaguchi et al., The Prostate in press). In the EAP model, we observed inflammation in the ventral prostate, while in the HCP model we observed inflammation in the lateral prostate. Consistent with previous studies, pelvic pain was observed in the EAP model. In addition, we found for the first time that HCP led to a significant increase in pelvic pain. Repeated treatment with tadalafil attenuated the pelvic pain and prostatic inflammation in both models. We observed that tadalafil would exhibit its pain attenuation effect through its anti-inflammatory action in the inflamed prostate.

Symposium

3-S25-4

Most recent findings of curative drugs for lower urinary tract symptoms in clinical practice

Yoshihisa Matsukawa[1], Yasuhisa Funabashi[1], Momokazu Gotou[1]

[1]Department of Urology, Nagoya University Graduate School of Medicine

Current guidelines for the treatment of benign prostatic hyperplasia (BPH) in several countries recommend the use of α_1-adrenoceptor antagonists (α1-blockers) or 5-alpha-reductase inhibitors (5ARIs), or phosphodiesterase 5 (PDE5) inhibitors for patients with lower urinary tract symptoms (LUTS) suggestive of BPH (LUTS/BPH). α1-blockers, such as tamsulosin and silodosin which are popular and frequently prescribed α1-blockers, improve LUTS/BPH by relaxing the smooth muscle tone of the prostate and bladder neck. 5ARIs, such as dutasteride and finasteride, were reported to improve LUTS by decreasing prostate size through inhibition of 5-AR. Meanwhile, tadalafil is the only PDE5-inhibitor approved for patients with LUTS/BPH. Smooth muscle relaxation in the bladder, urethra, and prostate due to increased nitric oxide/cGMP pathway activity via inhibition of PDE5 isoenzymes was reported to be the mechanism of LUTS improvement with tadalafil. Additionally, The use of an anticholinergic agent or a β3-adrenergic receptor agonist should be considered in patients with overactive bladder symptoms (overactive bladder symptom score ≥6).
In this symposium, I would like to introduce most recent findings of these curative drugs for LUTS in clinical practice.

Symposium

3-S26-1

Neuroprotective effects of the peel of *Citrus kawachiensis* (Kawachi Bankan) and auraptene in the hippocampus of hyperglycemia mice and global cerebral ischemia/reperfusion injury mice.

Satoshi Okuyama[1], Atsushi Sawamoto[1], Mitsunari Nakajima[1], Yoshiko Furukawa[1]

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The peel of *Citrus kawachiensis* (Kawachi Bankan), a citrus species grown in Ehime, Japan, is abundant in auraptene. Hyperglycemia and brain ischemia induce inflammation and oxidative stress and cause massive damage in the brain; therefore, we examined the anti-inflammatory and other effects of the dried peel powder of *C. kawachiensis* in the type 2 diabetic db/db mice model and global cerebral ischemia mice. The *C. kawachiensis* treatment inhibited astroglial activation in the hippocampus and the hyperphosphorylation of tau protein in hippocampal neurons, and also relieved the suppression of neurogenesis in the dentate gyrus of the hippocampus in the db/db mice. The *C. kawachiensis* treatment inhibited microglial and astroglial activation, and neuronal cell death in the hippocampus of transient global cerebral ischemia mice. It was suggested that the dried peel powder of *C. kawachiensis* exerts anti-inflammatory and neuroprotective effects in the brain. Auraptene, a coumarin compound, have been shown to exert anti-inflammatory effects in peripheral tissues; therefore, we attempted to demonstrate the effect in the brains in streptozotocin-induced hyperglycemic mice and transient global cerebral ischemia mice. Auraptene administration showed the similar effects as the peel of *C. kawachiensis* in the hippocampus of these mice models. These results suggested that auraptene have potential effects as a neuroprotective agent in the peel of *C. kawachiensis*.

Symposium

3-S26-2

Anti-diabetic effect of ethanol extract of *Cyclolepis genistoides* D. Don (palo azul), made in Paraguay

Hiromi Sato[1], Asami Funaki[1], Yuki Kimura[1], Mai Sumitomo[1]

[1]Clinical pharmacology and Pharmacometrics, Grad. Sch. of Pharmaceut. Sci, Chiba Univ.

Extract of *Cyclolepis genistoides* D. Don (vernacular name palo azul; palo) is a traditional medicine used in Paraguay for diabetes and is sold in Japan as dietary supplement. This study aimed to elucidate the mechanism of anti-diabetes activity of palo, especially focused on insulin resistance. Palo promoted adipocytes differentiation and regulated adipokine profiles in 3T3-L1 adipocytes by modulation of PPARγ, a major regulator of adipose differentiation. While palo didn't affect insulin signaling molecules, it promoted GLUT4 (glucose uptake transporter) translocation from cytoplasm to plasma membrane and increased 2-DG uptake under insulin stimuli. Human adipocyte showed almost similar profile with 3T3-L1 against palo treatment. In addition, as the other insulin targeted cell, effect on muscle differentiation was examined. Palo increased differentiation of C2C12 mouse muscle myoblasts and increased 5'-AMP-activated protein kinase (AMPK) activation. Finally palo treatment (250 or 1000 mg/kg) was performed with C57BL/6J mice for 14 weeks, being fed high-fat-diet (HFD60) simultaneously. Palo 250 mg/kg exhibited a tendency to decrease adipose volume but with increase of PPARγ mRNA expression, and decreased blood glucose level (P=0.058). Put it all together, palo has a potential to have antidiabetic effect by modulating insulin resistance via PPARγ pathway.

Symposium

3-S26-3

Anti-diabetic effects of citrus flavonoids on pancreatic β-cell function.

Yukiko Kaneko[1], Tomohisa Ishikawa[1]

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To promote β-cell function and survival would provide therapeutic approaches to prevent the onset and development of type 2 diabetes. Nobiletin, a citrus flavonoid, is known to improve hyperglycemia and insulin resistance in type 2 diabetic model mice. However, little is known about the effect of these citrus flavonoids on β-cells. In the present study, we investigated the effects of citrus flavonoids on insulin secretion from pancreatic β-cells and β-cell apoptosis. Nobiletin, a polymethoxylated flavone found in the peels of citrus fruits, significantly increased glucose-induced insulin secretion (GSIS) at 10 and 100 μM. In addition, nobiletin at 10 μM significantly inhibited thapsigargin-induced apoptosis of INS-1 cells. At this concentration, nobiletin significantly potentiated forskolin-induced cAMP accumulation in INS-1 cells. Sudachitin, a 5,7,40-tridesmethyl nobiletin derivative, also significantly increased GSIS at 100 μM and slightly inhibited β-cell apoptosis, although the effects of sudachitin were less potent than those of nobiletin. These findings suggest that citrus flavonoids facilitates GSIS and prevents ER stress-mediated β-cell apoptosis. Thus, citrus flavonoids may be used as a novel agent for the prevention of type 2 diabetes by promoting both survival and function of β-cells.

Symposium

3-S26-4

Molecular mechanism underlying metabolic beneficial effects of Citrus sudachi, a specialty of Tokushima area.

Licht Miyamoto[1], Koichiro Tsuchiya[1]

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Sudachi (Citrus sudachi) is a small sour citrus. It grows exclusively in Tokushima region of Shikoku island, but is a typical seasoning for Japanese fish cuisine. We have demonstrated that repetitive administration of crude sudachi peel extends life span in diabetic model rats, and successfully identified a couple of molecules and fractions which are beneficial for our health in terms of energy metabolism from peels of sudachi. A limonene-derivative, which turned out to facilitate expression of a longevity gene, sirt1, exhibited lipid-lowering effects in HFD-fed mice as well as in cultured cells, for instance. Our recent results on the metabolic effects of such components in sudachi peel will be shared in the current symposium.

Symposium

3-S27-1
Developmental History of Sublingual Immunotherapy

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Allergen specific immunotherapy is the only curative treatment for IgE-mediated allergic diseases in contrast to symptomatic treatment such as anti-histamine agents. Subcutaneous immunotherapy (SCIT) has been introduced in Japan for treatment of allergic rhinitis and/or asthma caused by pollens and/or house dust mites (HDM) in early 1960s, and the clinical efficacy has been well-known. However, the major drawbacks of SCIT are necessity of repeated painful injections as well as the risk of severe systemic adverse reactions. Sublingual immunotherapy (SLIT) was developed to resolve these issues. In Japan, Japanese cedar (JC) pollen SLIT-drop was developed initially for treatment of JC pollinosis, and approved for patients of 12 years of age and older in 2014. For adolescent and adult patients with HDM-allergic rhinitis, HDM SLIT-tablet was launched in 2015 and subsequently approved to be also available for pediatric patients (<12 years of age) in 2018. Moreover, JC pollen SLIT-tablet for JC pollinosis was approved in 2018 for all patients with no age limit. In this symposium, we present the development of SLIT drop/tablets including the current understanding on the mechanism of action.

Symposium

3-S27-2

Mechanisms of allergen immunotherapy elucidated through integrated comparative analysis

Osamu Kaminuma[1,2], Minoru Gotoh[2,3], Kimihiro Okubo[2,3], Akihiro Nakaya[2,4], Takachika Hiroi[2]

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Allergen immunotherapy (AIT) is an effective treatment for allergic rhinitis, although a substantial proportion of the patients is refractory. We aimed to elucidate the mechanisms underlying the effectiveness of AIT. A 2-year clinical study was performed in adult patients with Japanese cedar pollinosis with sublingual administration of cedar pollen extract. After dividing high-responder (HR) patients with improved severity scores and non-responder (NR) patients with unchanged or exacerbated symptoms, differences in HR and NR patients were evaluated by analyzing peripheral blood cellular, serum, and genetic profiles before and after the AIT. This treatment was highly effective for rhinitis symptoms, though unimproved clinical responses were seen in ~30% of the treated patients. Serum cytokine bead array analysis failed to distinguish NR from HR patients, though cluster analysis of the serum parameters revealed a positive correlation between Th1/Th2 cytokines in HR patients before and after the AIT. In the expression of a taste receptor in $CD4^+$ T cells, a copy number variation-related difference was observed between HR and NR patients. Through the pathway analysis of $CD4^+$ T cell-expressing genes, an apoptosis pathway was implicated in the efficacy of AIT. $CD4^+$ T cell is a predominant target of AIT to exhibit its efficacy on allergic rhinitis.

Symposium

3-S27-3

The analysis of Th2 cell subsets in house dust mite allergic rhinitis patients after sublingual immunotherapy

Fumie Ihara[1], Daiju Sakurai[2], Yoshitaka Okamoto[2]

Th2 cells are well known to play important roles in allergic diseases including allergic rhinitis (AR). Meanwhile, the factors that induce and sustain the pathogenesis of AR remain unclear. The recent development of sublingual Immunotherapy (SLIT) is expected to allow changes to the underlying pathogenesis of AR. However, the phenotype of the pathogenic Th2 cells (Tpath2) cells in house dust mite-induced AR (HDM-AR) and the relation between Tpath2 and SLIT efficacy have not been clarified. Therefore we analyzed the cytokine production and frequency of HDM-reactive T-cell subsets in peripheral blood mononuclear cells (PBMCs) using flow cytometry in 89 HDM-AR patients (placebo; n = 43 and HDM 300 IR; n = 46) who participated in a placebo-controlled study of SLIT with HDM tablets. All patients provided samples both before treatment as a baseline and at the end of the 52-week study. HDM-reactive IL-5^{+}IL-13^{+}CD27^{-}CD161^{+}CD4^{+} cells and ST2^{+}CD45RO^{+}CD4^{+} cells were observed in the PBMCs from each patient with HDM-AR; these cells significantly decreased after SLIT in the group treated with active tablets. HDM-reactive ST2^{+}CD45RO^{+}CD4^{+} cells were significantly lower in active-responders.

In conclusion, HDM-reactive ST2^{+}CD45RO^{+}CD4^{+} cells or those combined with IL-5^{+}IL-13^{+}CD27^{-}CD161^{+}CD4^{+} cells may be useful as markers indicating the successful treatment of SLIT. These cells may play a crucial role in the pathogenesis of HDM-AR as Tpath2.

Symposium

3-S27-4

Analyses of Foxp3$^+$ Treg cells and Tr1 cells in subcutaneous immunotherapy (SCIT)-treated allergic individuals in humans and mice

Masaya Matsuda[1], Tetsuya Terada[2], Kazuyuki Kitatani[1], Ryo Kawata[2], Takeshi Nabe[1]

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Mechanisms of allergen immunotherapy have not been fully elucidated. We have analyzed whether numbers of Foxp3$^+$ Treg cells and Tr1 cells (IL-10-producing Foxp3$^-$ CD4$^+$ T cells) are increased in SCIT-treated Japanese cedar pollinosis patients and allergic mice. Peripheral blood mononuclear cells (PBMCs) were collected from the patients treated with or without SCIT. Ovalbumin (OVA)-sensitized mice received s.c. dosages of OVA for SCIT, followed by intratracheal challenges with OVA. The lungs were collected from the allergic mice treated with or without SCIT. The human PBMCs and murine lung cells were stimulated, and analyzed by flow cytometer. In both strains, numbers of Tr1 cells but not Foxp3$^+$ Treg cells in SCIT-treated individuals were significantly larger than those in the non-SCIT-treated. In mice, SCIT treatment ameliorated allergic airway inflammation such as eosinophilia, hyperresponsiveness and histological changes. In another experiment of mice, Tr1 cells were induced in vitro by culture of splenocytes of sensitized mice with OVA and cytokines, and adoptively transferred to sensitized mice, resulting in effective suppression of the allergic airway inflammation. In conclusion, Tr1 cells could play roles in clinical effectiveness of SCIT.

Symposium

3-S28-1

Inhibition of the development of retinal degenerative diseases via regulation of microRNA function

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A microRNA (miRNA) is a kind of a small non-coding RNA functioning RNA silencing and post-transcriptional regulation of gene expression. The changes in expression of various miRNAs are reported to be associated with diseases, such as cancer, neurodegenerative diseases, and so on.
Glaucoma and retinitis pigmentosa (RP) are the leading causes of blindness in adults. Excitotoxicity caused by excess glutamate in the retinal extracellular space is thought to be one of the mechanisms of retinal ganglion cell death induced by glaucoma. RP is characterized by progressive photoreceptor-selective degeneration, and caused by mutation of the genes related to the function of photoreceptor and retinal pigment epithelium.
Recently, we demonstrated that the expression levels of some miRNAs were changed in the murine retina challenged excitotoxicity and that of a hereditary RP model animal. Retinal neurodegeneration in the NMDA-injected eye and the hereditary RP model animal could be reduced by intravitreal or subretinal injection of the molecules that modulate miRNA function, such as miRNA mimics and miRNA inhibitors. These results suggest that the development of retinal degenerative diseases could be inhibited by regulation of microRNA function.

Symposium

3-S28-2

Dysregulation in glial function causes pathogenesis of glaucoma

Youichi Shinozaki[1], Kazuhiko Namekata[2], Kenji Kashiwagi[3], Nobuhiko Ohno[4,5], Akiko Takeda[1], Takayuki Harada[2], Schuichi Koizumi[1]

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Glaucoma is a leading cause of blindness worldwide. Although an elevated intraocular pressure (IOP) is considered to damages retinal ganglion cells (RGCs) thereby causing blindness, it has become apparent that many risk factors other than IOP are involved in the etiology of glaucoma. Recent genome wide association studies (GWAS) have identified that single nucleotide polymorphism (SNP) of *ABCA1* gene is the highest risk for glaucoma. However, its pathogenic mechanisms are totally unclear. To address this issue, we analyzed molecular mechanisms using conventional ABCA1 knockout (KO) mice. We found that deficiency of ABCA did not increase IOP levels regardless of their age. Importantly, ABCA1KO mice at middle-age (12 months old) showed significant increases in the number of apoptotic RGCs. We also found that ABCA1 was enriched in astrocytes. To further clarifying the role of astrocytic ABCA1, we generated astrocyte-specific ABCA1 knockout (cKO) mice. The cKO mice had no IOP elevation and increased the number of apoptotic RGCs. The cKO mice also showed impaired visual functions at middle-age. Taken together, our data showed that (1) ABCA1 has no impact on IOP; (2) loss-of-function of ABCA1 is involved in glaucoma; and (3) ABCA1 in glial cells contributes to pathogenesis of glaucoma.

Symposium

3-S28-3

Regeneration therapy for retinal degeneration using iPS cell-derived retinal tissue

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Retinitis Pigmentosa is a group of hereditary diseases with rod photoreceptors degeneration, which is followed by central cone degeneration in the advanced stage. The patients experience night blindness and the progressive loss of visual field. Since the retinal bipolar cells that receive signals from the dying photoreceptors remain for a while, one therapeutic approach is to supply the photoreceptors to reconstruct initial visual transmission to these bipolar cells. We previously showed that mouse ES or iPS-derived retinas can develop structured photoreceptor layers after transplantation and the proximal presence of pre- and post-synaptic markers of the host photoreceptors and the graft bipolar cells were confirmed either by immunohistochemistry or genetic labeling. Evident light responsive activities of host retinal ganglion cells (RGC) were recorded by multiple electrode array system (MEA) over the grafted area in the retinas of end-stage retinal degeneration mice (rd1) that has only few remaining activities. Behavior test results also suggested the recovery of light perception in these mice after transplantation. Human ES/iPS retinas can also develop photoreceptor layers after transplantation in immune-deficient end stage retinal degeneration models such as NOG-rd1 or rhodopsin mutant SD-Foxn1 Tg(S334ter)3LavRrrc nude rats with some synaptic contact between the host and the graft by immunohistochemistry. We are preparing clinical trials using iPS-derived retinas.

Symposium

3-S28-4
Modulation of visual representation in the brain

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Vision is the sense we most depend on in our daily lives to interpret our surroundings. Visual information from the world enters the eye and reaches the retina. Ganglion cells in the retina send the information via the lateral geniculate nucleus to the primary visual cortex (V1). Neurons in V1 extract simple local features such as oriented lines and edges, by responding selectively to lines or edges with a particular slope (orientation selectivity). The V1 sends its output to a hierarchical series of higher visual areas, which represent a variety of higher order visual features, including motion, image segmentation, and object recognition. Impairment of the visual pathway at any stage will potentially cause blindness. Thus, modulating the visual pathway could be a therapeutic target of visual impairment. Acetylcholine modulates neuronal activities in various brain regions to control brain functions including attention, memory, and cognition. Here we determined the effect of donepezil, an acetylcholinesterase inhibitor, on visual recognition by examining the visual detection task and V1 activity in mice.

Symposium

3-S29-1
Involvement of RAN translation products and neurodegeneration in a triplet repeat disease, FXTAS.

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In pathological mechanism of repeat diseases, "RNA toxicity" is observed, which repeat-derived RNA binds to various RNA binding proteins, thereby causing abnormalities in RNA metabolism. In addition to its RNA toxicity, "RAN translation" has been found, in which repeat RNA initiates translation in a start codon (AUG) independent manner. In many neurological diseases caused by abnormal elongation of repeat sequences, polypeptides derived from repeat RNA are accumulated in cells. In fragile X-related tremor/ataxia syndrome (FXTAS), which is a triplet repeat disease, elongated CGG repeats in the 5 untranslated region of FMR1 mRNA elicits RAN translation, polyglycine-containing protein (FMRpolyG). In this study, we analyzed the binding protein with FMRpolyG and investigated its involvement with FXTAS pathology. Using immunohistochemistry, FMRpolyG was observed in mouse A9 monochromosomal hybrid cells that harbor whole human X chromosome derived from FXTAS, but not in cells derived from a normal individual. In order to identify binding proteins to FMRpolyG, cell lysates of mouse A9 cells carrying the X chromosome from FXTAS were immunoprecipitated using FMRpolyG antibody, and shotgun proteomics analysis was performed. As a result, it was revealed that causative molecules of some repeat diseases and exosome-related proteins bind to FMRpolyG. These results suggest that we can clarify the mechanisms involved in the onset of other neurological diseases through research on FXTAS.

Symposium

3-S29-2

Animal models of synucleinopathies: prion-like propagation of alpha-synuclein in wild-type animals

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Accumulation of insoluble alpha-synuclein (aS) is a pathological hallmark of some neurodegenerative diseases including Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy, collectively termed synucleinopathies. aS is deposited in a hyperphosphorylated and ubiquitinated form with β-sheet-rich fibrillar structure in diseased brain. A growing body of evidence suggests that spreading of aS pathology occur by prion-like propagation mechanisms. Our study revealed that intracerebral injection of insoluble aS into wild-type mice can induce prion-like propagation of phosphorylated aS pathology even at 1 month after injection, while injection into aS knockout mice failed to induce any pathologies. We also have demonstrated that intracerebral injection of insoluble aS into adult common marmoset resulted in spreading of aS pathologies and neurodegeneration. These in vivo experiments clearly indicate that insoluble aS has prion-like properties and it propagates through neural networks. The underlying mechanisms of aS propagation are poorly understood, however, aS propagation model animals would be useful in elucidating pathogenetic mechanisms and developing disease-modifying drugs for synucleinopathies.

Symposium

3-S29-3

Sleep abnormality as a potential target of disease-modifying therapy for neurodegenerative diseases

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Sleep abnormality is a prevalent but under-recognized symptom affecting patients with various neurodegenerative diseases including Alzheimer's disease (AD) and Parkinson's disease (PD) from the early stage of the diseases. Sleep abnormality of these patients was conventionally attributed to AD or PD pathology that affects the brain regions regulating sleep-wake or circadian rhythm. On the contrary, various epidemiological studies have demonstrated the association of sleep abnormality with an increased risk of AD or PD. However, relevant disease models to prove the causal relationship between sleep abnormality and neurodegenerative diseases were lacking.
We recently succeeded in inducing chronic sleep abnormality closely resembling that of AD patients in AD model mice and revealed that chronic sleep fragmentation, a specific subtype of sleep abnormality frequently observed in the patients of neurodegenerative diseases, indeed exacerbates AD pathology in the mice brain. Our findings are in accord with previous epidemiological studies in humans and thus would contribute to the understanding of the underlying pathomechanisms and informing the development of disease-modifying therapy for neurodegenerative diseases.

Symposium

3-S29-4

Efficient exploration of preventive drugs against spinocerebellar ataxia using cultured cerebellar Purkinje cells

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Neurodegenerative diseases are caused by progressive degeneration of specific neurons. To overcome neurodegenerative diseases, the exploitation of preventive drugs is strongly expected, since impaired neurons are not regenerated by drugs. Spinocerebellar ataxia (SCA) is a group of dominantly-inherited neurodegenerative diseases. SCA is classified into SCA1-47 by the variance of causal genes. Since SCA patients are commonly characterized by cerebellar ataxia and atrophy of cerebellum, it is possible that there are common pathogenic mechanisms in SCAs. However, we can not find any shared functions among SCA-causing proteins. We have explored the molecular pathogenesis of several SCA-causing proteins. Especially, we have constructed in vitro SCA model to express these mutant proteins in primary cultured cerebellar Purkinje cells (PCs), which are characterized highly-branched dendrites and are important for cerebellar functions. Several SCA-causing mutant proteins commonly impair dendritic development of PCs. We assume that this phenomenon is one of the common phenotypes of SCA in vitro. This SCA model would be useful for the efficient exploration of novel preventive drugs against various types of SCAs.

Symposium

3-S30-1

Study on colorectal cancer using a human colorectal air liquid interface organoid model

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Tumor microenvironment has been implicated in tumor development and progression. As a three-dimensional tumor microenvironment model, air liquid interface (ALI) organoid culture system was recently produced. In our previous study, ALI organoid model from normal and tumor colorectal tissues of human patients was established. Both organoids were successfully generated and showed cystic structures containing an epithelial layer and surrounding mesenchymal stromal cells. Structures of tumor organoids closely resembled primary tumor epithelium. Tumor organoids were more resistant to toxicity of 5-fluorouracil and Irinotecan than colorectal cancer cell lines, SW480, SW620 and HCT116 (Usui et al., *Stem Cells Int*, 2016, *Curr Protoc Toxicol*, 2018). We also demonstrated that Hedgehog signals mediate anti-cancer drug resistance in colorectal tumor patient-derived ALI organoids and that the inhibitors are useful as a combinational therapeutic strategy against colorectal cancer (Usui et al., *Int J Mol Sci*, 2018).

Symposium

3-S30-2
Role of PPARG, a Transcriptional Factor on Hypertension

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Peroxisome proliferator-activated receptor gamma (PPARG) is a ligand-activated transcription factor regulating metabolic and vascular function. We previously reported that mice (S-DN) expressing dominant-negative PPARG in smooth muscle cells (SMC) are hypertensive, exhibit impaired vascular relaxation, and display reduced expression of a novel PPARG target gene, RhoBTB1. We hypothesized that RhoBTB1 may play a protective role in vascular function that is disrupted in S-DN mice and in other models of hypertension. We then generated double transgenic mice (termed S-RhoBTB1) with tamoxifen-inducible, Cre-dependent expression of RhoBTB1 in SMC. S-RhoBTB1 mice were crossed with S-DN to produce mice (S-DN/S-RhoBTB1) in which tamoxifen-treatment restored RhoBTB1 expression in aorta to normal. Increased blood pressure (BP) and impaired vasodilation in S-DN were reversed by restoration of RhoBTB1 in SMC. To test if RhoBTB1 can prevent angiotensin (Ang) II-induced hypertension, Ang II (490 ng/min/kg) was infused in tamoxifen-treated S-RhoBTB1 for two weeks. Ang II-induced increased BP and impaired vasodilation were blunted in S-RhoBTB1. We conclude that a novel PPARG target gene, RhoBTB1, functions in SMC to facilitate vasodilation and mediates a protective anti-hypertensive effect.

Symposium

3-S30-3

The role of dietary iron supplementation in rat nonalcoholic steatohepatitis model.

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Nonalcoholic fatty liver disease (NAFLD) is now the major chronic liver disease in the world. Iron overload occurring in CLDs is a risk factor for the disease progression; however, it is still unclear whether iron overload contributes to the progression of NAFLD to nonalcoholic steatohepatitis (NASH). In this study, we investigated the pathological role of iron overload in a rat NASH model. Six-week-old male F344 rats were fed a control, high-fat (HF), high-fat high-iron (HFHI) and high-iron (HI) diets for 30 weeks. Rats in HF and HFHI groups showed an ALT-dominant elevation of serum transaminases, hepatic steatosis, and inflammation with upregulation of proinflammatory cytokines. In HFHI group, the number of inflammatory foci, equivalent to lobular inflammation in NASH patients, significantly increased with upregulation of inflammatory cytokines such as TNF-α, compared to HF group. Macrophages laden with iron were seen in the inflammatory lesion of HFHI group. These results suggested that dietary iron supplementation enhances experimental steatohepatitis induced by long-term HF diet feeding. Excessive activation of macrophages stimulated by iron accumulation can be involved in the exacerbation of inflammation.

Symposium

3-S30-4
The role of IL-19 in liver fibrosis

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Nonalcoholic fatty liver disease (NAFLD) is highly associated with the metabolic syndrome, and occurs as a more serious form of the disease, nonalcoholic steatohepatitis (NASH). NASH is diagnosed pathologically by histological evaluation of fibrosis, inflammation, and other features, such as hepatocyte ballooning. IL-19 is a member of the IL-10 family and is an anti-inflammatory cytokine produced mainly by macrophages. The last 10 years from the finding of IL-19, investigations underline the role of IL-19 in the immunological diseases such as inflammatory bowel disease, pancreatitis, and contact hypersensitivity. However, the involvement of IL-19 in liver inflammation and liver fibrosis is not well understood. We investigated the immunological role of IL-19 in NAFLD/NASH model mice fed by a choline-deficient and high-fat diet with methionine and cholesterol for 2 months. IL-19 knockout mice (KO) showed the major feature of NASH such as pericellular fibrosis, although wild-type mice showed steatohepatitis. In this symposium, we will report the recent advances in the role of IL-19 as inflammatory mediators in NASH. IL-19KO may be a valuable tool to study the NAFLD/NASH.