3-S28-1 Symposium28

Overview of recent GPCR research

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G protein-coupled receptors (GPCRs) receive a wide range of extracellular stimuli, and transduce their information into the cells. As the first step, GPCR bind agonist. Then, GPCRs change their conformation to transduce information of agonists through transmembrane. However, GPCRs do not directly activate intracellular signaling molecules. GPCR-mediated signaling always requires G proteins to generate second messengers. GPCR research starts from analysis of the tissue responses by agonist stimulation. Then, GPCRs are labeled and quantified by radioactive ligands. It revealed heterogeneity of GPCRs that we have imaged before. Nucleotide sequences of GPCRs are subsequently determined and function of GPCRs are analyzed by gene mutation, overexpression and knockout. Application of imaging techniques to GPCR research reveals interaction with accessary proteins, dimerization, and so on. Structures of GPCRs are finally determined by X-ray analysis. Although crystallization of GPCR has resolved many important issues of GPCRs, there are many unresolved issues such as structural basis of G protein selectivity and drug design based on structural information. I will overview history and progress of GPCR research.

Shedding light on complexity of G-protein coupling by large-scale functional assays

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Signal transduction initiated from GPCRs is primarily mediated by heterotrimeric G proteins, which are grouped into four families (Gs, Gi, Gq and G12). G-protein-coupling pattern determines a cellular response unique to each GPCR and thus profiling these patterns is a critical step toward understanding GPCR biology. However, due to functional redundancy of multiple members and signal cross talk downstream of G-protein signaling, it is challenging to assess coupling signature of every G-protein member at a large scale. Over years, our lab has spent efforts to establish and standardize GPCR tools that enable measurement of individual G-protein coupling and signaling. These include a panel of GPCR effector-KO cells (G-protein, β-arrestin, GRK), TGFα shedding assay, NanoBiT-G-protein dissociation assay, etc. By combining these tools, we have recently profiled G-protein coupling of ～150 GPCRs across all G-protein members. Bioinformatics analysis of the large-scale dataset allowed us to identify generic GPCR residues that are involved in G-protein-coupling selectivity. Using the coupling-featuring residues, we generated a predictor algorithm that scores G-protein coupling based on an amino-acid sequence. Intriguingly, we successfully designed a DREADD that switched coupling from Gq to G12. In this symposium, we will explain our GPCR tools and present a recent advance in understanding where and how G-protein-coupling selectivity is encoded by "coupling determinants" in GPCRs. We will also present assays to measure G12, the least characterized G-protein family, and will discuss potential drug targets for G12-related diseases.

Time-resolved Conformational Analysis during GPCR-Gs Coupling

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Protein-protein interactions and conformational changes of a signaling protein are major mechanisms of cellular signal transduction. To understand the precise signaling mechanism, studies have investigated the structural mechanism of signaling proteins using various biochemical and/or biophysical techniques such as X-ray crystallography, nuclear magnetic resonance (NMR), electron microscopy, and electron paramagnetic resonance. In addition to these techniques, surface labeling mass spectrometry has been successfully used for conformational analysis of signaling proteins. Exposed or flexible regions have higher labeling rates and buried or ordered regions have lower labeling rates. Although surface labeling mass spectrometry does not provide 3D structural information, it analyzes dynamic protein conformations that are difficult to be analyzed with other techniques. GPCR signal transduction involves extensive protein-protein interactions and conformational changes of related signaling proteins. In this seminar, I will discuss the conformational mechanisms of GPCR signaling analyzed by surface labeling mass spectrometry.

TAK-071, a novel M_1 positive allosteric modulator with low cooperativity, improves cognitive function in rodents with few cholinergic side effects

Haruhide Kimura

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The muscarinic M_1 receptor (M_1R) is a promising target for treating cognitive impairment associated with cholinergic deficits. We found that cooperativity (α-value) was key to lowering the risk of diarrhea by M_1R positive allosteric modulators (M_1 PAMs), and discovered a low α-value M_1 PAM, TAK-071 with α-value of 199 and inflection point (IP) of 2.7 nM. T-662, a reference M_1 PAM with high α-value of 1786 and IP of 0.62 nM, but not TAK-071, augmented isolated ileum motility. TAK-071 and T-662 improved scopolamine-induced cognitive deficits in rats at 0.3 and 0.1 mg/kg, respectively, and induced diarrhea at 10 mg/kg and 0.1 mg/kg, respectively, in rats. TAK-071 might have a wider margin between cognitive improvement and diarrhea induction than T-662. M_1R activation increases neural excitability via membrane depolarization, reduced afterhyperpolarization, and generation of afterdepolarization in prefrontal cortical pyramidal neurons. T-662 induced all three processes, whereas TAK-071 selectively induced afterdepolarization. Combining sub-effective doses of TAK-071, but not T-662, with an acetylcholinesterase inhibitor, significantly ameliorated scopolamine-induced cognitive deficits in rats. TAK-071 may therefore provide new therapeutic opportunities for cognitive dysfunction with minimum cholinergic side effects.

Development of protein-ligand binding prediction method by deep learning

Masakazu Sekijima

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Virtual screening is a promising computational method for obtaining novel hit compounds in drug discovery. It aims to enrich potentially active compounds from a large chemical library for further biological experiments. However, the accuracy of current virtual screening methods is insufficient.

Drug discovery requires to find molecules that interact with targets with high affinity and specificity. Virtual screening application has been developed to this goal. However, current methods still show relatively weak predictive power.

In this work, we proposed a new virtual screening method named Visual Inspection Network (VisINet) inspired by image classification. We will show how recent advances in computer vision can be applied to structure-based virtual screening by adopting a CNN model based on the ResNet architecture.

Current status and future perspectives in cardiovascular and neural toxicity testing using human iPSC technology

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Unexpected toxicities, such as cardiotoxicity and neurotoxicity, is one of the key issues for failure of novel drug candidates in development. Human iPSC technology hold great promise as in vitro models to study the pharmacological effects of drug candidates. Based on international efforts by CiPA (Comprehensive In Vitro Proarrhythmia Assay) and JiCSA (Japan iPS cardiac safety assessment), in vitro safety studies using iPSC-cardiomyocytes have demonstrated their ability to inform on drug-induced delayed repolarization and proarrhythmic risk. Notably, the new assay methodologies, such as iPSC-cardiomyocytes and in silico, has been discussed at ICH S7B/E14 from last autumn. CNS toxicity testing forms a part of the "core battery" of safety pharmacology. Drug-induced seizure is a major reason for drug attrition. Currently, human iPSC-neurons are expected to evaluate seizure liability by multi-electrode assay system by the non-profit worldwide organization HESI (NeuTox subteam). In addition to drug safety issue, developmental neurotoxicity (DNT) testing, which has been discussed at OECD, is focusing on iPSC technology by providing mechanistic data at the cellular and molecular levels.
Thus, iPSC technology is becoming a promising tool for various safety/toxicology fields. In the symposium, I would like to provide an overview of current status of the use of iPSC-derived cardiomyocytes and neural cells in toxicity testing and discuss future perspectives.

3-S29-2 Symposium2

Analysis of contractile functions of human iPS-derived cardiomyocytes using motion field imaging

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Human iPS cell-derived cardiomyocyte (hiPSC-CM) is conceptually promising as an unlimited source of human cardiomyocytes for cardiac pharmacological assessment including pre-clinical safety testing. However, intra- and interline variation in functional properties of hiPSC-CM remain to be solved completely. In order to improve the accuracy of pharmacological assessment, we conducted a multidisciplinary approach for developing new methods to evaluate effects of drugs on contractile functions. We aimed to increase throughput of pharmacological assessment for contractile functions of hiPSC-CMs using a motion field imaging which is a noninvasive assay system using high speed video image of hiPSC-CMs. The technique enabled us to obtain precise and stable quantitative values for contractile functions of hiPSC-CMs from single cells, and revealed a relationship between contractile function and molecular expression in hiPSC-CMs. The relationship was consistent with what we investigated in murine cardiac cells. We would like to discuss how the multidisciplinary approach can improve predictability of pharmacological/toxicological assessment for physiological functions of hiPSC-CMs. (JSPS KAKENHI JP17K19499, JP19H03380, AMED 19mk0104117)

High throughput screening in silico via computer models of induced-pluripotent stem cell derived cardiomyocytes

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There is a profound need to develop a strategy to predict patient-to-patient vulnerability in the emergence of cardiac arrhythmia. A promising in vitro method to address patient-specific proclivity to cardiac disease utilizes induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs). A major strength of this approach is that iPSC-CMs contain donor genetic information and therefore capture patient-specific genotype-phenotype relationships. A cited detriment of iPSC-CMs is the cell-to-cell variability observed in electrical activity. We postulated, however, that cell-to-cell variability may constitute a strength when appropriately utilized in a computational framework to build in silico cell populations that can be employed to identify phenotypic mechanisms and pinpoint key sensitive parameters. Thus, we have exploited variation in experimental data across multiple laboratories to develop a computational framework to investigate subcellular phenotypic mechanisms in healthy iPSC-CMs. In subsequent studies, this computational framework was utilized to explore genotype-phenotype relationships in control and diseased cases.

3-S29-4 Symposium29

Personalized medicine diagnostics for cardiac safety

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Induced Pluripotent Stem Cell and Electrophysiology Laboratory Office of Science and Engineering Laboratories Center for Devices and Radiological Health US Food and Drug Administration

Personalized medicine uses a diagnostic test to predict which patients will benefit and which are likely to be harmed by therapies. Induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) could potentially be utilized in personalized cardiotoxicity studies, assessing individual proarrhythmic risk. We compared subject - specific iPSC - CMs responses to dofetilide and moxifloxacin with individual clinical responses to the same drugs for a cohort of 16 healthy normal subjects. The delay in iPSC-CMs repolarization and QT interval prolongation induced by these hERG potassium channel blockers were used as surrogate end points of proarrhythmic risk. Comparative results showed no significant correlation between the subject - specific in vitro repolarization prolongation slopes and clinical QT response slopes to either moxifloxacin (P = 0.75) or dofetilide (P = 0.69). Similarly, no significant correlation was found between baseline QT and baseline APD measurements (P = 0.93). This result advances our current understanding of subject - specific iPSC - CMs and facilitates discussion into factors obscuring correlation and considerations for future studies of subject - specific iPSC-CMs assays used to predict individual clinical outcome.

3-S29-5 Symposium2

Modeling of psychiatric disorders using iPSC technology

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iPSC technology has enabled us to more accurately model the pathology of human diseases in drug discovery research. Schizophrenia is characterized by positive symptoms such as hallucinations and delusions, negative symptoms such as blunted affect and social withdrawal and cognitive dysfunction. Previous studies have suggested that the abnormal development of neuronal cells, impaired synaptic functions, and impaired neural circuit functions are the causes of psychiatric disorders, however, much remains unknown about the molecular and cellular etiology of these disorders. To analyze the molecular and cellular etiology of psychiatric disorders and to identify the molecular mechanisms behind the inter-individual variability of response to antipsychotics, we established iPSCs from patients with schizophrenia for which there is existing clinical information, such as treatment history describing their responsiveness to antipsychotic drugs, and differentiated these iPSCs into neurons. As an example, we found evidences suggesting that differential synaptic function is a potential candidate for the molecular basis of response to antipsychotics. Pathological studies using clinical information and iPSC-derived neurons from patients can be powerful for understanding the molecular and cellular etiology of psychiatric disorders.

3-S30-1 Symposium30

Serotonergic activation and antidepressant-like effects

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Numerous studies have suggested that the activation of central serotonergic systems are involved in several emotional/cognitive changes including anxiogenic, antidepressant, and anti-impulsive effects. If each effect is regulated by distinct serotonergic systems, more efficient and safer treatments of depression would become possible at least theoretically. To this end, we examined the effects of selective manipulations of serotonergic activity in each brain region and specific serotonin receptor on emotional/cognitive functions by using recently developed optogenetic tools and serotonin receptor knockout mouse. We used an elevated plus-maze test, a forced swim test, and a 3-choice serial reaction time task to assess anxiety-, antidepressant-like behavior, and impulsive action, respectively. Our results demonstrated that serotonergic activity in the dorsal raphe nucleus has a pivotal role in antidepressant-like effects and anti-impulsive effects, but not anxiogenic effects while that in the median raphe nucleus regulates anti-impulsive and anxiogenic effects, but not antidepressant-like effects. Furthermore, our results suggest that the activation of dorsal raphe nucleus - ventral tegmental area/substantia nigra serotonergic pathway would exert antidepressant-like effects without affecting anxiety or impulsivity.

Novel mode of antidepressant action based on exercise-induced beneficial effects

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Major depression is a highly prevalent mental disorder affecting many people worldwide. Although selective serotonin reuptake inhibitors (SSRIs) are the most widely used antidepressants, a significant proportion of depressed patients do not achieve remission after initial treatment. It has been known that physical exercise provides neurogenic and antidepressant effects, and we recently demonstrated that the serotonin type 3 (5HT3) receptor is essential for exercise-induced hippocampal neurogenesis and antidepressant effects. In this study, we examined the 5HT3 receptor-mediated mechanism underlying hippocampal neurogenesis and antidepressant effects, and tried to establish novel prevention and treatment for depression, which is based on mechanisms of exercise-induced beneficial effects. Here, we showed that treatment with a 5HT3 receptor agonist induces antidepressant effects and increases hippocampal neurogenesis, in a fluoxetine-independent manner. In addition, histological analyses revealed that the 5HT3 receptor and insulin-like growth factor 1 (IGF1) are expressed in the same neurons in the hippocampal dentate gyrus. Furthermore, in vivo microdialysis and drug microinjection analyses showed that 5HT3 receptor agonist treatment increases extracellular IGF1 levels in the hippocampus, and that IGF1 signaling is required for the 5HT3 receptor-dependent hippocampal neurogenesis. Our findings suggest a novel 5HT3 receptor-IGF1 mechanism that is distinct from fluoxetine-induced responses. A novel mode of antidepressant action could provide a new therapeutic target for depression, especially bringing significant benefits for SSRI-resistant depressed patients.

Role of prefrontal VEGF signaling in the rapid antidepressant actions of ketamine

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Previous studies have shown that the NMDA receptor antagonist ketamine produces rapid and sustained antidepressant effects in treatment-resistant depressed patients, and that brain-derived neurotrophic factor (BDNF) signaling in the medial prefrontal cortex (mPFC) mediates the antidepressant actions of ketamine. Recently, we have found that the antidepressant-like effects of ketamine are blocked by forebrain excitatory neuron-specific deletion of either vascular endothelial growth factor (VEGF) or its receptor Flk-1, intra-mPFC infusion of a VEGF neutralizing antibody, or local knockdown of Flk-1 in mPFC excitatory neurons. Intra-mPFC infusion of VEGF is sufficient to produce ketamine-like behavioral actions, which are blocked by neuron-specific Flk-1 deletion. Moreover, inhibition of neuronal VEGF signaling blocks the neurotrophic/synaptogenic effects of ketamine. These findings indicate that neuronal VEGF-Flk-1 signaling in the mPFC plays a key role in the antidepressant actions of ketamine. We have also demonstrated that a heterologous interplay between BDNF and VEGF signaling in the mPFC is required to produce ketamine-like antidepressant responses to these neurotrophic factors. Together, these findings provide evidence for the neurotrophic mechanisms underlying the rapid and sustained antidepressant actions of ketamine, and pave the way for the development of rapid and more effective antidepressants with fewer side effects than ketamine.

3-S30-4 Symposium30

Looking into glial power in regulation of mood disorders

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It is now well understood that astrocytes intimately interact with neurons to support and regulate their functions in many aspects. One of most discussed functions of astrocytes concerns their role in extracellular potassium spatial buffering. A wealth of investigations has focused on the astroglial-neural interactions at the tripartite synapses, where astrocyte processes tightly wrap around pre- and post-synaptic sites. In contrast, not as much attention has been placed on astroglial-neural interaction in proximity to neuronal soma. Particularly, how astrocytes regulate intrinsic firing patterns of neurons, and what structural basis may underlie this regulation, are much less explored. Here we demonstrated the level of Kir4.1 on astrocytes tightly regulates the degree of membrane hyperpolarization and the amount of burst activity of lateral habenular (LHb) neurons. Astrocyte-specific overexpression of Kir4.1 in LHb drives more neuronal bursting and causes depressive-like symptoms. Conversely, knocking down Kir4.1 or overexpression of its dominant negative form in LHb reduces neuronal bursting and ameliorates behavioral despair and anhedonia. Together, we revealed a new form of glial-neural interaction in setting neuronal firing mode in a devastating psychiatric disease, and discover the therapeutic potential of targeting LHb Kir4.1 for treating major depression.

3-S31-1 Symposium31

Myocardial atrophy regulated by the formation of TRPC3 protein complex

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Myocardial atrophy, characterized by the decreases in size and contractility of cardiomyocytes, is caused by severe malnutrition and/or mechanical unloading. We have investigated the mechanism underlying induction of myocardial atrophy induced by anti-cancer drug treatment, and found that formation of protein complex between NADPH oxidase 2 (Nox2) and transient receptor potential canonical (TRPC) 3 contributed to ROS-mediated myocardial atrophy in mice. Here, we report that extracellular adenosine 5'-triphosphate (ATP) promotes nutrient deficiency-induced cardiomyocyte atrophy through TRPC3-Nox2 complex formation. Knockdown of either TRPC3 or Nox2 suppressed nutritional deficiency-induced ATP release, as well as ROS production and NRCM atrophy, suggesting that the formation of TRPC3-Nox2 protein complex amplifies ATP-induced myocardial atrophy. Taken together, we propose that TRPC3-Nox2 axis mediates nutritional deficiency-induced cardiomyocyte atrophy by promoting ATP release.

Interstitial mesenchymal progenitors are crucial for homeostatic skeletal muscle integrity

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Sarcopenia, comprising the loss of skeletal muscle mass and strength, constitutes an important health problem associated with adverse outcomes such as disability, poor quality of life, and even death. Decline in muscle strength precedes the loss of muscle mass in older adults, suggesting decreased muscle quality as causal factor of sarcopenia. One of the notable changes in muscle quality during aging is the increase in fat infiltration, which is attributable to interstitial mesenchymal progenitors. However, the precise mechanism by which these cells contribute to sarcopenia remains unknown. Here we show the essential role of mesenchymal progenitors in the maintenance of steady state skeletal muscle by generating mesenchymal progenitor-depleted mice. Specific ablation of mesenchymal progenitors led to phenotypes markedly similar to sarcopenia including muscle weakness, myofibre atrophy, fibre type alteration, and denervation at neuromuscular junctions. Through searching for genes responsible for mesenchymal progenitor-dependent muscle maintenance, we found that bone morphogenetic protein 3b (Bmp3b) is specifically expressed in mesenchymal progenitors whereas its expression level is significantly decreased by aging or adipogenic differentiation. The functional importance of Bmp3b in maintaining muscle mass was demonstrated by using knockout mice and cultured muscle cells treated with recombinant BMP3B. Furthermore, administration of BMP3B to aged mice resulted in improved energy metabolism and an increase in muscle mass and strength. These results reveal previously unrecognized mechanisms whereby interstitial mesenchymal progenitors ensure muscle integrity and suggest that age-related changes of mesenchymal progenitors contribute to sarcopenia. Our study highlights a critical role of stromal components to sustain parenchyma, raising the possibility of the broader importance of such mesenchymal-parenchymal interactions in diverse tissue homeostasis.

A system for evaluating the contractile force of myotubes, and drug discovery applications

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Aging and/or disuse of muscles result in losses of muscle mass and function. Muscle atrophy affects not only an individual's quality of life but also healthy life expectancy with increased risks of various diseases. Recent epidemiological studies revealed a significant correlation between muscle mass and lifespan. The prevention of muscle atrophy and the maintenance of muscle mass based on exercise therapies and with the development of new drugs are thus receiving increased attention. Skeletal muscle cells (myotubes) are frequently used in basic research. Evaluations of myotubes' quality are generally undertaken by measuring the diameter/area and the expression level of contractile proteins such as myosin heavy chain protein, but these indicators do not directly express the cell quality. The contractile force is a better indicator of myotube quality. However, an appropriate method for evaluating cells' contractile force has not been established.

This presentation introduces a system that our group recently developed for measuring the cell contraction force. With this method, myotubes are cultured on a two-layer silicone substrate of differing hardness. When the myotubes are stimulated by an electric pulse, the muscle contraction force is visualized as wrinkles. The length of the wrinkles is correlated with the magnitude of the force, and thus the values of the wrinkles can be used as an indicator of force. This system will contribute to the discoveries of new drugs designed to prevent or improve muscle atrophy.

Role of mechanosensing machinery in muscle regeneration

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Skeletal muscle has the capacity to regenerate myofibers after muscle injury. Upon a variety of stimuli including mechanical stretch, muscle-resident stem cells called muscle satellite cells (MuSCs) are committed to become myoblasts that can fuse with each other to generated multinucleated myotubes. We previously reported that transbilayer relocation of phosphatidylserine is essential for morphogenesis of myotubes through the function of PIEZO1, a mechanosensitive cation channel that is activated by membrane tension. However, the molecular entity that determines the cell fate of MuSCs remains to be elucidated. In this session, we will present our recent data showing that mechanosensitive ion channels play crucial roles in activation of MuSCs. *In silico* analysis demonstrates that at least three mechanosensitive ion channels including PIEZO1 are strictly expressed in MuSCs. By genetic elimination, we revealed that those mechanosensitive ion channels have distinct roles during myogenesis, suggesting that cell fate of MuSC is largely dependent on mechanosensation through a triad of mechanosensitive ion channels.

3-S32-1 Symposium32

Development of antibody-drug conjugates that target vascular endothelial cells to promote anti-tumor activity

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Antibody drugs have revolutionised and strongly impacted therapy in the 21st century, especially in the field of cancer and inflammation-related diseases. Moreover, the development of bispecific antibodies as well as antibody-drug conjugates forms a new platform of antibody drugs by extending application range and by increasing efficacy. Although anti-cancer therapies pay much attention to the induction of cancer cell death, the cancer microenvironment and the functional roles of vascular endothelial cells in metastasis are also important to understand to regulate the growth of cancer cells.

We observed that anti-high mobility group box-1 antibody (HMGB1 Ab) was incorporated into vascular endothelial cells in culture under certain conditions. Moreover, Alexa Fluor 488 labeled anti-HMGB1 Ab was distributed and accumulated specifically in the vascular endothelial cells of melanoma inoculated in mice whereas not accumulated in normal vascular endothelial cells in the brain, lung and liver. Based on these results, we hypothesized that anti-HMGB1 Ab can be utilized as the anti-tumor drug carrier to improve the efficiency for cancer treatment. Therefore, we biosynthesized anti-HMGB1 Ab-doxorubicin conjugates and injected it into the melanoma-bearing mice. The tumor growth was significantly suppressed in HMGB1 Ab-doxorubicin conjugates treatment group compared with the group treated with doxorubicin alone. In conclusion, anti-HMGB1 Ab may function as a drug delivery molecule targeting the cancer vascular endothelial cells.

S100A8/A9 and its novel receptors play a critical part in organ tropic cancer metastasis

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Cancer cells frequently show organ specific metastasis, in which the "seed and soil" theory has long been proposed. In this concept, it has first reported that S100A8/A9, a heterodimer complex composed of S100A8 and S100A9 proteins, which exhibits a " soil signal", produced and secreted from the lung in the tumor-bearing body works as a strong ligand to a soil sensor, TLR4, presented on cancer cells, in which the S100A8/A9-TLR4 binding leads cancer cells to metastasize the lung area where S100A8/A9 is abundant. In addition, RAGE was also included as a soil sensor for the S100A8/A9-mediated lung tropic cancer metastasis. Besides to these receptors, we recently showed the presence of novel soil sensors for S100A8/A9, namely; EMMPRIN, NPTN, ALCAM and MCAM. We then referred to these collections of receptor proteins as "novel S100 Soil Sensor Receptors (novel SSSRs)". In this presentation we hence introduce a crucial role of S100A8/A9-novel SSSRs axis on cancer metastasis. The bindings of S100A8/A9 to individual SSSRs play an important part in cancer metastasis through upregulation of cellular motility and invasiveness of cancer cells. To take the metastatic force away from cancer cells, we developed novel biologics that prevent interaction of S100A8/A9 with SSSRs, and are followed by efficient suppression of the S100A8/A9-mediated lung tropic metastasis *in vivo*.

3-S32-3 Symposium32

Development of anti-cancer agent targeting on (pro)renin receptor

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We previously reported that silencing of the *PRR* gene, which encodes the (pro)renin receptor ((P)RR) significantly reduced Wnt/β-catenin-dependent development of pancreatic ductal adenocarcinoma(PDAC), colorectal cancer and glioblastoma. Here, we examined the effects of a panel of blocking monoclonal antibodies (mAbs) directed against the (P)RR extracellular domain on proliferation of the human PDAC cell lines PK-1 and PANC-1 in vitro and in vivo. We observed that four rat anti-(P)RR mAbs induced accumulation of cells in the G0/G1 phase of the cell cycle and significantly reduced proliferation in vitro concomitant with a significant reduction in the expression of active β-catenin and cyclin D1. Systemic administration of the anti-(P)RR mAbs to nude mice bearing subcutaneous PK-1 xenografts significantly decreased tumor expression of active β-catenin and the proliferation marker Ki-67 and reduced tumor growth. In contreffects ast, treatment with the handle region peptide of (pro)renin did not inhibit tumor growth in vitro or in vivo, indicating that the effects of the anti-(P)RR mAbs was independent of the renin–angiotensin system. These data indicate that mAbs against human (P)RR can suppress PDAC cells proliferation by hindering activation of the Wnt/β-catenin signaling pathway. Thus, mAb-mediated (P)RR blockade could be an attractive therapeutic strategy for PDAC and other cancers.

Potential involvement of the mitochondrial unfolded protein response in depressive-like symptoms in mice

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Many evidences strongly suggest that a mitochondrial deficit is implicated in major depression. A mitochondrial deficit leads to mitochondrial stress responses, including the mitochondrial unfolded protein response (UPRmt), which is associated with certain brain disorders such as spastic paraplegia and Parkinson's disease. However, there is no evidence regarding the relationship between depressive disorder and UPRmt. Mice treated with chronic restraint stress showed significant depressive-like behaviors in the tail suspension and forced swim tests. In addition, the isolated brain mitochondria showed decreased oxygen consumption rate, decreased protein expressions related to oxidative phosphorylation, and increased levels of molecules associated with UPRmt, such as Hspa9, Hspd1, Ubl5, Abcb10, and ClpP. The expressions of all of the UPRmt-related molecules were significantly correlated with depressive-like behavior in the forced swim test. Thus, the present study is the first to reveal a relationship between the UPRmt and depressive disorder, suggesting that the UPRmt is a potential drug target for depressive disorders.

The function of intracellular muscarinic acetylcholine receptor: influence of physiological stress

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Brain acetylcholine is an important neurotransmitter to regulate synaptic transmission in neural circuits and to enhance learning, memory and cognition. Acetylcholine released from nerve terminals acts on muscarinic and nicotinic acetylcholine receptors on plasma membrane followed by the rapid hydrolytic degradation. We found that muscarinic acetylcholine receptors M1 (M1-mAChRs) are highly expressed on intracellular membranes, such as endoplasmic reticulum and Golgi apparatus, in neurons of central nervous system and activate signaling cascades distinct from those of cell surface receptors. The intracellular M1-mAChRs is activated by endogenous acetylcholine following its uptake *via* a putative transport system and causes cholinergic facilitation of synaptic long-term potentiation in the hippocampus. In addition, the cholinergic synaptic regulation is found to disappear after chronic restraint stress. We herein introduce function of intracellular M1-mAChR in the brain and discuss about possible relation to stress-related neuropsychiatric disorder.

Neural mechanisms involved in the physical stress-induced inhibition of ovarian function

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Stress is known to change the secretion of ovarian steroid hormones via the hypothalamic-pituitary-ovarian (HPO) axis. Noxious physical stress can cause reflex responses in visceral function via autonomic nerves. In this symposium, I will talk about our recent animal studies on neural mechanisms involved in ovarian estradiol secretion induced by noxious physical stress stimulation. In anesthetized rats, noxious physical stress decreased ovarian estradiol secretion. Electrical stimulation of the ovarian sympathetic nerves (superior ovarian nerves: SON) decreased ovarian estradiol secretion. Sympathectomy or spinal transection was effective for disrupting the physical stress-induced estradiol decrease responses, while decerebration was ineffective. Thus, the inhibition of ovarian estradiol secretion during physical stress was mainly integrated in the brainstem, and this inhibitory response was due to reflex activation of the ovarian sympathetic nerves. The sympathetic inhibitory regulation of ovarian estradiol secretion was pronounced when the HPO axis was inhibited by chronic estradiol treatment in rats. Considering the female life cycle, extensive physical stress may inhibit ovarian function, especially before puberty and during old ages when the HPO axis is inactive.

Central regulation mechanisms for brain nicotinic acetylcholine receptor-mediated activation of the sympatho-adrenomedullary outflow

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During exposure to stress, the stress-related information is conveyed to the brain, which recruits neuronal and neuroendocrine systems, thereby inducing physical and behavioral responses including activation of the sympatho-adrenomedullary (SA) system for adaptation to stress. This system is essential for adaptation to stressful conditions, while excessive or sustained exposure to stress can play a pathogenic role in triggering and sustaining a variety of diseases such as hypertension and arrhythmia via excessive or sustained activation of the SA system. Therefore, it is necessary to clarify "central" regulation mechanisms for the SA outflow to elucidate fundamental mechanisms for development of these diseases in response to stress. We have investigated central regulation mechanisms for the SA outflow focusing on brain nicotinic acetylcholine receptors (nAChRs), because stress can increase smoking and (-)-nicotine, a major component of cigarette smoke, is reported to exert hypertension via not only peripheral but also central nAChRs. In this presentation, we will introduce our data showing central regulation mechanisms for brain nAChR-mediated activation of the SA outflow, focusing on brain prostanoids, cannabinoid receptors and nitric oxide.

Diverse roles of nitric oxide synthases in the pathogenesis of lung diseases

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To a greater or lesser extent, all three isoforms of nitric oxide synthases (nNOS, iNOS, and eNOS) are expressed in the lung under both physiological and pathological conditions. Although the role of NOSs in lung diseases has been examined in pharmacological studies with non-selective NOSs inhibitors, it still remains to be fully elucidated due to non-specificity of the agents. We addressed this point in our mice lacking all three NOSs and in mouse lung disease models. Bleomycin treatment significantly increased pulmonary fibrosis, collagen content in the lung, and the total cell number in bronchoalveolar lavage fluid in wild-type (WT), single $nNOS^{-/-}$, $iNOS^{-/-}$, and $eNOS^{-/-}$, and triple $n/i/eNOSs^{-/-}$ mice as compared with saline treatment. Those changes were significantly exacerbated only in the triple $NOSs^{-/-}$ mice, but not in any single $NOS^{-/-}$ mice, as compared with the WT mice, suggesting a protective role of NOSs in the development of pulmonary fibrosis (*Respir Res* 2014). Chronic hypoxic exposure significantly caused an increase in right ventricular pressure, right ventricular hypertrophy, and pulmonary artery remodeling in all the WT, single $NOS^{-/-}$, and triple $NOSs^{-/-}$ mice as compared with normoxic exposure. Those alterations were significantly aggravated in the triple $NOSs^{-/-}$ mice and, to a lesser extent, in the $eNOS^{-/-}$ mice as compared with the WT mice, again suggesting a protective role of NOSs in the development of pulmonary hypertension (*AJRCCM* 2018). In contrast, ovalbumin-induced bronchial thickening, eosinophilic infiltration, and mucus secretion were markedly mitigated in the triple $NOSs^{-/-}$ than in the WT mice, suggesting an opposing injurious role of NOSs in the development of bronchial asthma (*Lung* 2016). These results suggest that, even in the same lung organ, the role of NOSs appears to be different in distinct disease conditions, demonstrating diverse roles of NOSs in the pathogenesis of lung disorders.

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Pharmacological aspects of Respiratory Diseases

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With the recent aging population, pneumonia, lung cancer, and chronic obstructive pulmonary disease (COPD) are leading death causes, and asthma and interstitial pneumonia are common. Suppression of drug-resistant bacteria has become a global topic, and one of the most interesting findings in Japan is macrolide therapy on several chronic respiratory diseases with its new effects other than antibacterial activity.

In asthma and COPD, inhalation medications have gained clinical benefits, but phenotype differences between Japanese and Western patients are focused. According to the Japanese guidelines for asthma-COPD overlap (ACO), proper diagnosis and treatment indication of various combinations of inhaled corticosteroids, long-acting β_2 agonists and muscarinic M_3 receptor antagonists will be focused.

Antifibrotic drugs are used in patients with idiopathic pulmonary fibrosis (IPF), and new drug discovery is progressing from more detailed knowledge of the mechanism of pulmonary fibrosis.

Pulmonary hypertension is common in both patients with interstitial pneumonia and COPD, but little treatment progress is seen compared to primary pulmonary hypertension. Advanced knowledge about diagnosing and treating this disease are expected.

I will introduce interesting topics of respiratory diseases from a pharmacological and clinical viewpoints and perspectives, in addition to the understanding of respiratory system and diseases.

Pathological mechanisms and novel therapeutic outlooks for sepsis-associated acute lung injury

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Sepsis affects every major organ within the body, ultimately leading to their failure, and the development of one or more organs poses a major threat to the survival of septic patients. In sepsis, the respiratory system is the most frequently affected organ system, and lung dysfunction is the first step in the development of multiple organ failure. A major hurdle in the clinical management of septic patients suffering from acute lung injury and its most severe manifestation, the acute respiratory distress syndrome, is the lack of the effective treatment. The important goal in critical care medicine is to find significant therapeutic strategies that will impact favorably on patient outcome. Sepsis alters expression of many pathogenic factors that can potentially give rise to abnormalities in the respiratory system. A number of transcription factors, such as nuclear factor-kappaB and activator protein-1, can be linked to the altered gene activation during sepsis. Thus, several transcription factors may play a pivotal role in the pathophysiology of acute lung injury in sepsis. Given that sepsis can be regarded as a gene-related disorder, the potential usefulness of systemic delivery of decoy oligodeoxynucleotide against some transcription factors may be considered to be a promising novel therapeutic approach for treatment of acute lung injury in sepsis.

Role of neuropeptides in lung

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Severe influenza infection is characterized by a strong inflammatory response and profuse viral replication. These viruses, such as H5N1 avian flu, have a high rate of death and to date there are no effective treatments. Cross-talk between the autonomic nervous system and the immune system by means of sympathetic and parasympathetic pathways is a critical process in host defense. Activation of the sympathetic nervous system results in the release of catecholamines (CA) as well as neuropeptide Y (NPY). Here we investigated whether phagocytes are capable of de novo production of NPY, as has been described for CA. We show that synthesis of NPY and its Y1-receptor (Y1R) were increased in phagocytes in lungs following severe influenza virus infection. Genetic deletion of Npy or Y1r specifically in phagocytes greatly improved the pathology of severe influenza virus infection, which is characterized by excessive virus replication and pulmonary inflammation. Mechanistically it is the induction of suppressor of cytokine signaling 3 (SOCS3) via NPY-Y1R activation that is responsible for impaired anti-viral response and promoting pro-inflammatory cytokine production, thereby enhancing the pathology of influenza virus infection. Thus, direct regulation of the NPY-Y1R-SOCS3 pathway on phagocytes may act as a fine-tuner of an innate immune response to virus infection, which could be a therapeutic target for lethal influenza virus infection.

Requirement for neuropeptide Y in the development of allergen-induced airway hyperresponsiveness and inflammation

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Neuropeptide Y (NPY) is a neurotransmitter that is widely expressed in the brain and peripheral nervous system. Various immune cells express the NPY Y1 receptor. NPY modulates these cells via its Y1 receptor; however, involvement of NPY in the pathophysiology of bronchial asthma, particularly airway hyperresponsiveness (AHR), has not been defined. NPY-deficient and wild-type mice were intranasally sensitized and challenged to house dust mite (HDM) extract, and airway responses were monitored. After sensitization and challenge, NPY-deficient mice showed significantly lower AHR than wild-type mice, and numbers of eosinophils and levels of type-2 cytokines [interleukin (IL)-4, IL-5, and IL-13] in bronchoalveolar lavage fluid were significantly lower. Type-2 cytokine production from splenic mononuclear cells of HDM-sensitized mice was also significantly lower in NPY-deficient mice. Treatment with a NPY receptor antagonist, significantly suppressed development of HDM-induced AHR and inflammation in wild-type mice. These data identify an important contribution of NPY to allergen-induced AHR and inflammation. Thus, manipulating NPY represents a novel therapeutic target to control allergic airway responses.

Non-coding RNAs and bronchial smooth muscle hyperresponsiveness in allergic bronchial asthma

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Non-coding RNAs, such as microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), play important roles in normal and diseased cell functions. A small GTPase RhoA is a key protein of bronchial smooth muscle (BSM) contraction, and an up-regulation of RhoA has been demonstrated in BSMs of experimental asthma. Our previous study also demonstrated that RhoA translation was controlled by a miRNA, miR-133a, in BSMs. In human BSM cells (hBSMCs), an up-regulation of RhoA was observed when the function of endogenous miR-133a was inhibited by its antagomir. Treatment of hBSMCs with interleukin-13 (IL-13) caused an up-regulation of RhoA and a down-regulation of miR-133a. In a murine experimental asthma, increased expression of IL-13 and RhoA and the BSM hyperresponsiveness were observed. Interestingly, the level of miR-133a was significantly decreased in BSMs of the diseased animals. These findings suggest that RhoA expression is negatively regulated by miR-133a in BSMs, and that the miR-133a down-regulation causes an up-regulation of RhoA, resulting in an augmentation of the contraction. Recent studies also revealed an inhibitory effect of lncRNA *Malat1* on the miR-133a function. Thus, lncRNAs/miRNAs might be key regulators of BSM hyperresponsiveness, and provide us a new insight into the treatment of airway hyperresponsiveness in asthmatics.

Th9 cells elicit bronchial hyperresponsiveness through eosinophil-independent mechanisms

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Th2 cells play an important role in the pathogenesis of bronchial asthma, because eosinophils migrated into the lungs in response to Th2 cytokines are considered to cause bronchial hyperresponsiveness (BHR). Recently, a newly identified T cell subset, Th9, was reported to induce the same responses as Th2 cells. We have demonstrated that antigen-induced airway eosinophil infiltration and BHR developed in mice transferred with Th9 cells, without an assistance of IgE/mast cell-dependent responses. However, differential mechanisms were implicated in BHR elicited by Th2 and Th9 cells. Th9 cell-mediated BHR was reproduced in eosinophil-deficient mice and resistant to glucocorticoid treatment, whereas Th2 cell-mediated BHR disappeared in those conditions. Identification of mediators responsible for not only eosinophil-mediated responses but also eosinophil-independent BHR may be useful for the development of new anti-asthma drugs, especially against steroid-resistant asthma.

Th2 cells induce nasal type-1-hypersensitivity-like reaction in mice.

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Background: Allergen-mediated cross-linking of IgE on mast cells and basophils are well-recognized trigger for type-1 allergic diseases such as allergic rhinitis (AR). However, allergens may not be the sole trigger for AR, and several allergic-like reactions are induced in non-IgE-mediated ways. In this study, we examined a non-IgE-mediated, endotoxin-triggered nasal type-1-hypersensitivity-like reaction in mice. **Methods:** To investigate whether endotoxin affect sneezing response, mice were intraperitoneally immunized with ovalbumin (OVA), then nasally challenged with endotoxin-free or endotoxin-containing OVA. Further, to investigate the role of T cells, mice were adoptively transferred with in vitro differentiated OVA-specific Th2 cells, then nasally challenged with endotoxin-free or endotoxin-containing OVA. **Results:** Endotoxin-containing, but not endotoxin-free, OVA elicited sneezing response in OVA-immunized FceRI-deficient mice. An OVA-specific Th2 adoptive transfer model demonstrated that local activation of antigen-specific Th2 cells was required for the response. **Conclusions:** We propose that antigen-specific nasal activation of CD4+ T cells followed by endotoxin exposure induces sneezing response.

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An exciting future of pharmacology

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Pharmacology is an energetic area of biomedical science, linking together chemistry, physiology and pathology, and crucial for discovering new drugs to help fight diseases. For the development of new drugs, advances in basic science must be achieved to create the seeds of new drugs. Such seeds must be translated into clinical trials at bedside. Thus, pharmacology requires the multidisciplinary integration of various levels of studies from bench to bedside and demands that we utilize a wide spectrum of methods to achieve our final goal. Pharmacology has adopted the latest developed technology immediately and raised the precision of not only the studies but also experimental therapeutics. We should discuss about the new exciting technique that is applied to a pharmacological study.

Pharmacology, drug discovery, and drug treatment: thinking about the present and future of AI

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As a representative of the JST ERATO Ikegaya Brain-AI Hybrid Project, I would like to take a bird's-eye view of recent trends in the application of machine learning in the pharmaceutical field and its surroundings, including my own research concepts. My ERATO team aims to establish a novel interdisciplinary field, namely, "Wisdom Engineering", which could ultimately revise our understanding of the functions of the brain and artificial intelligence (AI) by elucidating how brains and AI can cooperate when brains and AI are directly connected (brain-AI hybrid). Based on three directions, i.e., AI-assisted brain function, brain-inspired AI designing, and brain-AI co-learning, we are exploring the potential and limit of the plasticity of brain-AI hybrids, thereby extending the functions of the brain and AI and expanding the definition of wisdom. Finally, we aspire to bring our research achievements closer to human society; that is, we aim to improve the productivity, health, welfare, and happiness of individuals by optimizing human behaviors and social structures.

Message to women scientists: Success in research comes when you refuse to give up

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Gender gap is still huge in Japan. In Global Gender Gap Report 2020 by the World Economic Forum, Japan ranked 121st of 153 countries and the worst of G7 countries. The percentage of women university students is close to that of men, whereas that of graduate students is nearly 1/3. Number of women researchers is only 16.2% in 2018 and the percentages of women presidents, professors, and associate professors are 4.7, 10.4 and 17.1%, respectively. To overcome this gender gap in the scientific research, leaders of each field in Japan need to aware of the fact and give equal chance to women scientists. In my research field, two international societies have accomplished gender equality in the number of board members and try to keep it in speakers and chairpersons at the meeting. In the recent special issue on Circadian Rhythms at Eur. J. Neuroscience, all 23 reviews are written by women authors. So, leaders should aware and act. For young women researchers, followings are my message; 1. Never give-up, do not quite, be patient and don't mind what other people say. 2. Take advantage of anything and anybody. 3. Willingly accept the requested works. Respond quick and politely. 4. Any experience is useful and will become a life asset. So, don't haste. 5. Try to be recognized not domestically but internationally.

Systems Biology of Mammalian Sleep/Wake Cycles ～Phosphorylation Hypothesis of Sleep～

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The detailed molecular and cellular mechanisms underlying NREM sleep (slow-wave sleep) and REM sleep (paradoxical sleep) in mammals are still elusive. To address these challenges, we first constructed a mathematical model, Averaged Neuron Model (AN Model), which recapitulates the electrophysiological characteristics of the slow-wave sleep. Comprehensive bifurcation analysis predicted that a Ca^{2+}-dependent hyperpolarization pathway may play a role in slow-wave sleep. To experimentally validate this prediction, we generate and analyze 26 KO mice, and found that impaired Ca^{2+}-dependent K^+ channels (*Kcnn2* and *Kcnn3*), voltage-gated Ca^{2+} channels (*Cacna1g* and *Cacna1h*), or Ca^{2+}/calmodulin-dependent kinases (*Camk2a* and *Camk2b*) decrease sleep duration, while impaired plasma membrane Ca^{2+} ATPase (*Atp2b3*) increases sleep duration. Genetical (*Nr3a*) and pharmacological intervention (PCP, MK-801 for *Nr1/Nr2b*) and whole-brain imaging validated that impaired NMDA receptors reduce sleep duration and directly increase the excitability of cells. Based on these results, we propose **phoshporylation hypothesis of sleep** that phosphorylation-dependent regulation of Ca^{2+}-dependent hyperpolarization pathway underlies the regulation of sleep duration in mammals. We also recently developed a simplified mathematical model, Simplified Averaged Neuron Model (SAN Model), which uncover the important role of K^+ leak channels in NREM sleep. In this talk, I will also describe how we identify essential genes (*Chrm1* and *Chrm3*) in REM sleep regulation, and propose a plausible molecular definition of a paradoxical state of REM sleep.