Title of Project: How are Synapses Formed, Fine-tuned and Eliminated in vivo?—Novel Mechanisms by the Complement Family Proteins

Michisuke Yuzaki
(Keio University, School of Medicine, Professor)

Research Project Number: 15H05772
Researcher Number: 40365226
Research Area: Neuroscience
Keyword: Neuron, Synapse, Neural circuit, Complement, Glutamate receptor

【Purpose and Background of the Research】
Neuronal synapses are generated, fine-tuned and eliminated from early development all the way to adulthood, in an activity-dependent manner. These processes are thought to play crucial roles in cognitive functions and certain neurodevelopmental and neuropsychiatric disorders.

A complement C1q belongs to the innate immune system and recognizes various non-self targets to be eliminated. Recently, proteins related to C1q (C1q family) are shown to regulate glucose and lipid metabolism. Furthermore, we found that Cbln1 and C1ql1, which belong to the C1q family, are crucial for synapse formation, maintenance and synaptic plasticity.

In this project, focusing on major neuronal circuits in the hippocampus and cerebellum, we aim to clarify how C1q family proteins regulate synapse morphology and its functions. In addition, we will clarify how C1q family proteins play new roles in coordinating multiple systems involving energy metabolism and brain functions.

【Research Methods】
The cerebellum is essential for motor coordination and motor memory. Its major input fibers (parallel and climbing fibers) require Cbln1 and C1ql1, respectively, for synapse integrity. On the other hand, Cbln1, Cbln4, C1ql2 and C1ql3 exert crucial functions in specific hippocampal synapses to mediate episodic memory. We will clarify molecular mechanisms by which these C1q proteins regulate specific synapses.

C1q proteins are upregulated by increased neuronal activities, inflammation and metabolic needs. We aim to clarify how C1q proteins are produced, secreted and bind to specific receptors to coordinate multiple systems (Fig. 1).

Finally, we will develop molecular tools that could regulate C1q signaling to modify synaptic functions in vivo.

【Expected Research Achievements and Scientific Significance】
By clarifying signaling mechanisms mediated by C1q proteins, we expect to obtain better understanding about how synapses are generated, fine-tuned and eliminated in vivo. The results of these studies are also expected to pave the way for new and better treatment of certain neuropsychiatric disorders.

【Publications Relevant to the Project】

【Term of Project】FY2015-2019

【Budget Allocation】135,800 Thousand Yen

【Homepage Address and Other Contact Information】
http://www.yuzaki-lab.org
Title of Project: Elucidation of Mechanisms Regulating Neural Stem/Progenitor Cell Fate

Yukiko Gotoh
(The University of Tokyo, Graduate School of Pharmaceutical Sciences, Professor)

Research Project Number: 15H05773 Researcher Number: 70252251
Research Area: Biological Sciences, Neuroscience
Keyword: Neural stem cell, Neocortex, Development, Chromatin

Purpose and Background of the Research
One of the fundamental questions in understanding tissue development is how multipotent progenitors/tissue stem cells give rise to various cell types in a defined order to achieve appropriate tissue organization. Neocortical neural stem/progenitor cells (NPCs) attract much attention since these cells give rise to neuronal and glial cell types in a developmental-stage dependent manner with striking precision and can be used as a model system to address this developmental issue. We have previously shown that polycomb group (PcG) complex and high mobility group A (HMG) proteins play pivotal roles in driving fate switches of NPCs during neocortical development (Hirabayashi et al. Neuron 2009; Kishi et al. Nat. Neurosci. 2012: Morimoto-Suzuki et al. Development 2014). Therefore, in this study, we aim to investigate how these proteins are regulated and how they control the fate of NPCs in a developmental-stage-dependent manner.

In contrast to embryonic NPCs that quickly and sequentially produce a variety of neural cell types in a limited time, adult NSCs have a very different mission. Namely, they have to produce the same sets of neural cell types for a very long time (a lifetime) with little changes in their differentiation potentials. Recently, we have identified an embryonic origin of adult NSCs residing in the subependymal zone (Furutachi et al. Nat. Neurosci. 2015). We therefore aim to investigate the molecular basis of differences between this embryonic “origin” of adult NSCs and other embryonic NPCs, particularly focusing on their differentiation potentials.

Research Methods
We will examine what mechanisms might regulate the target specificity of PcG in a developmental time-dependent manner by focusing on the neurog1 and fezf2 loci. We will examine cis and trans elements necessary for PcG targeting to these loci.

Our previous work (Kishi et al., Nat. Neurosci. 2012) revealed that HMG proteins mediate the open chromatin state in early-stage NSCs, conferring the neurogenic potential on them. We will thus investigate the genome-wide regulation of the chromatin state in NPCs. We will isolate the embryonic origin of adult NSCs and compare their features with other embryonic NPCs.

Expected Research Achievements and Scientific Significance
We hope that our work will shed light on general mechanism of stem cell fate control.

Publications Relevant to the Project


Term of Project: FY2015-2019

Budget Allocation: 143,000 Thousand Yen

Homepage Address and Other Contact Information:
http://www.f.u-tokyo.ac.jp/~molbio/
Title of Project: Transcriptional Regulation by TGF-β Signaling and its Relation to Progression of Cancer

Kohei Miyazono  
(The University of Tokyo, Graduate School of Medicine, Professor)

Research Project Number: 15H05774  Researcher Number: 90209908
Research Area: Tumor biology
Keyword: Signaling, Biochemistry, Cancer microenvironment, Cancer stem cell, Genome research

<table>
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<tr>
<th>Purpose and Background of the Research</th>
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<td>Transforming growth factor-β (TGF-β) was identified in the early 1980s as a factor that induces the anchorage-independent growth of normal fibroblasts. TGF-β was subsequently found to inhibit the growth of most types of normal cells, including epithelial cells and lymphocytes. In the mid-1990s, TGF-β was shown to induce the epithelial-mesenchymal transition (EMT) in certain epithelial cells. TGF-β is now known to exhibit bi-directional effects, i.e. tumor-suppressive effects and tumor-promoting effects, during cancer progression (figure below).</td>
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In this project, we will investigate how TGF-β loses its tumor-suppressive effects and acquires tumor-promoting effects during cancer progression. We will uncover the molecular mechanisms underlying the bi-directional effects of TGF-β on tumors using next-generation DNA sequencers, analyze EMT cell phenotypes using proteins and/or genes expressed in the cells undergoing EMT, and identify some TGF-β targets with therapeutic potential for certain types of cancer.

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<td>The biological importance and complicated mechanisms of action of TGF-β make them especially interesting to study. Much effort has been devoted to developing antagonists against TGF-β family proteins and their receptors, and some are in clinical trials. However, a better understanding of the TGF-β signaling pathways would facilitate the clinical development and application of such antagonists. The EMT is involved in the invasion and metastasis of cancer, but the underlying mechanisms are still not fully understood. Drugs that regulate the EMT have yet to be developed, and such drugs may be useful for treatment of certain types of cancer in the future.</td>
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[Research Methods]

Project 1. Investigate dynamic changes in the transcriptional machinery as regulated by the TGF-β-Smad pathways.

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[Expected Research Achievements and Scientific Significance]

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[Publications Relevant to the Project]


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[Homepage Address and Other Contact Information]

http://beta-lab.umin.ac.jp
Title of Project: Studies in Structural Physiology of Channels

Yoshinori Fujiyoshi
(Nagoya University, Graduate school of Pharmaceutical Sciences/CeSPI, Professor)

Research Project Number: 15H05775 Researcher Number: 80142298
Research Area: Biology
Keyword: Structural Biology

**[Purpose and Background of the Research]**


**[Expected Research Achievements and Scientific Significance]**

Advancing crystallography and IBSA, we will elucidate how channels work and regulate functions of the human body. We are intensively studying and advancing the interesting research field of Structural Physiology.

**[Publications Relevant to the Project]**


Crystal structure of a Claudin provides insight into the architecture of tight junctions.


Structural insight into tight junction disassembly by Clostridium perfringens enterotoxin.


**[Term of Project]** FY2015-2019

**[Budget Allocation]** 138,500 Thousand Yen

**[Homepage Address and Other Contact Information]**

http://www.cespi.nagoya-u.ac.jp/
Title of Project: Endomembrane-Mediated Organ Straightening and Defense in Plants

Ikuko Hara-Nishimura
(Kyoto University, Graduate School of Science, Professor)

Research Project Number: 15H05776  Researcher Number: 00241232
Research Area: Biology, Basic Biology, Plant Cell Biology
Keyword: Environmental responses, Organelle, Plant-microbe interaction, Plant molecular biology

Purpose and Background of the Research
After bending in response to gravity or light, plant organs have the ability to stop bending and resume straight growth. Remarkably, nothing is known about the fundamental molecular mechanisms responsible for this response. Our recent studies indicate that straightening is driven by an actin-myosin XI cytoskeleton in a specific type of cell having a long actin cables. The first aim of this project is to disclose the molecular mechanism of straightening of plant organs. Our previous findings suggest a model whereby dynamic interactions between endoplasmic reticulum (ER) and actin-myosin XI cytoskeleton determine the architecture and movement patterns of the ER strands. We also found an ER-derived organelle and designated it as ER body. ER bodies develop in epidermal cells in seedlings and accumulate a large amount of b-glucosidases, which can produce substances that potentially protect against invading pests. The second aim of this project is to unveil an ER-body function as a novel defense system in Brassicaceae plants.

Expected Research Achievements and Scientific Significance
Evidence from this research will provide an idea that plants have evolved the endomembrane-mediated strategies against biotic and abiotic stresses. This will give a valuable insight into the fields of plant tropism and defense.

Research Methods
Specific aims are: (1) to genetically identify factors responsible for straightening, to analyze actin dynamics, and finally to show that long actin filaments act as a bending sensor to trigger the straightening system and (2) to address how the ER bodies support the innate immunity against herbivores.

Publications Relevant to the Project
Title of Project: Contribution of Opsin Properties to Non-Visual Functions

Akihisa Terakita
(Osaka City University, Graduate School of Science, Professor)

Research Project Number: 15H05777  Researcher Number: 30212062
Research Area: Animal physiology and behavior
Keyword: Animal physiological chemistry, Photobiology

【Purpose and Background of the Research】
Most animals capture light with light-sensitive proteins, opsins and utilize light information for vision and non-visual functions such as light-regulation of biological rhythms. Hence, light-sensing physiologies start with opsin light-absorption; molecular properties of opsin are considered to relate to characteristics of light sensing in cells and organs. Therefore, it is important to understand how much contribution opsin properties make to light sensing functions of animals.

Pineal organs in lower vertebrates can sense not only dark and light but also the ratio of ultraviolet (UV) light and visible light, namely “color.” We previously found that the opsins involved in the pineal “color” detection were distinct from visual opsins and possess different molecular properties compared with those of visual opsins. Furthermore, one of the pineal opsin also expresses in the teleost parapineal organs, which are essential to form an asymmetric structure of the habenular nucleus, a part of brain, during its early development. In this study, we investigate physiological functions of “color” and UV detections in the pineal and parapineal organs, respectively, and also the contribution of opsin properties towards achievement of these functions.

【Research Methods】
We prepare and use mutant zebrafish in which the pineal opsins are replaced with visual opsins; in addition, we used opsin-gene knockout (KO) zebrafish. We measure the neural activities in the zebrafish brain by calcium imaging to investigate where in the brain and how the “color” information is transmitted. On the basis of the obtained results, we speculate relevant functions and conduct behavior tests with opsin-gene KO zebrafish. We also investigate contribution of pineal opsin properties in achieving the related functions by using opsin-replaced zebrafish. In addition, we also use the same mutants as described above to investigate how UV information captured in the parapineal organs transmits to the brain and whether the UV information contributes to forming habenular asymmetric structure.

【Expected Research Achievements and Scientific Significance】
It is expected that findings of this study could contribute to understanding biological meanings of “color” sensing in extraocular organs and also the physiological relevance of UV information in non-visual photoreception. In addition, relationship between light and brain development could provide a new insight into not only basic biology but also applied biological science.

【Publications Relevant to the Project】

【Term of Project】 FY2015-2019
【Budget Allocation】 134,400 Thousand Yen
【Homepage Address and Other Contact Information】
http://www.sci.osaka-cu.ac.jp/biol/mphys/
Title of Project: Molecular Mechanisms of Color Pattern Formation in Mimicry Controlled by Supergene

Haruhiko Fujiwara
(The University of Tokyo, Graduate School of Frontier Sciences, Professor)

Research Project Number: 15H05778  Researcher Number: 40183933
Research Area: Basic biology: Evolutionary biology
Keyword: Evolutionary genetics, Morphological evolution, Comparative genomics

[Purpose and Background of the Research]
The color patterns observed in various animals work as protective mimicry, but the formation mechanisms are largely unknown. Some complex and adaptive traits such as mimicry are controlled hypothetically by “supergene” which is composed of flanking genes, but there is little evidence for the hypothesis. Females of the swallowtail butterfly, Papilio polytes mimic color patterns and behavior of the unpalatable butterfly, Pachliopta aristolochiae (Fig. 1). We found that this Batesian mimicry is caused by a 130kb chromosomal region, which is fixed by chromosomal inversion. This region includes dsx involved in sexual differentiation and two additional genes, all of which possibly constitute supergene. To certify the idea, we aim to reveal (1) structure and function of the supergene, (2) mechanisms for emergence and stabilization of the supergene unit, and (3) structural comparison of supergene in the related species. We also clarify the mechanisms of mimicry caused by genome rearrangements, such as gene multiplication and transposition.

[Research Methods]
Focusing on the responsible region for Batesian mimicry in Papilio polytes (Fig. 2), we reveal the following points: (1) gene functions and expression profiles for dsx and two other genes in the supergene unit by a novel functional analysis (EMST method); (2) detailed structures of inverted regions in mimetic and non-mimetic chromosomes; (3) structures and functions of dsx and flanking genes in the closely related species to Papilio polytes. In addition, we also reveal the mechanisms of regulation for pupal protective coloration and larval marking pattern formation in Lepidoptera, which help to understand how the genome rearrangement contributes to emergence of the supergene unit.

[Expected Research Achievements and Scientific Significance]
This project aims to reveal general structure and functions of supergene which is an unclarified concept in genetics for a long period. We have recently developed a novel functional analysis, which enables detailed functions of all candidate genes in a supergene unit involved in mimicry. This unique approach gains the lead to other projects for supergene. We anticipate that our project clarifies how supergene has been formed, evolved and stabilized during evolution, which will impact on evolutionary genetics and evolutionary biology in general.

[Publications Relevant to the Project]

[Term of Project] FY2015-2019

[Budget Allocation] 153,800 Thousand Yen

[Homepage Address and Other Contact Information]
http://www.idensystem.k.u-tokyo.ac.jp/index.html
haruh@k.u-tokyo.ac.jp
Blast disease caused by the ascomycete fungus *Magnaporthe oryzae* is not only the most devastating disease of rice but also belongs to the group of seven most destructive diseases/pests of crops worldwide (Pennisi, E. 2010, Science). Breeding of resistant rice cultivars is the most cost-effective way to control the disease. Blast pathogen secretes a battery of effector proteins into rice cells to facilitate its invasion. It is hypothesized that effectors perturb host resistance and metabolism, paving the way for pathogens to establish in the host environment. However, their molecular functions are largely unknown. Certain effectors are recognized by host surveillance system, by the products of *R* (*Resistance*)-genes, in most cases coding for cytosolic Nucleotide-Binding Leucine Rich Repeat Receptors (NLRs). Effectors recognized by *R*-gene products are called avirulence effectors (AVRs). We cloned three AVRs, *AVR-Pia*, *AVR-Pii* and *AVR-Pik*, from the blast pathogen, as well as isolated their cognate *NLR* genes *Pia* and *Pii* from rice. *Pik* was isolated by Ashikawa and colleagues (Ashikawa et al. 2008, Genetics). Notably, all the three NLRs are composed of a pair of proteins encoded by two tightly linked genes. In this project, we will investigate the structure and function of the three AVRs, as well as elucidate their molecular interactions with NLRs. We will also address how the each NLR pair functions to trigger resistance.

**Research Methods**

We will attempt to identify the target proteins in rice for the three AVRs of *M. oryzae* and to understand their functions. AVR-target protein interactions as well as AVR-NLR interactions will be studied at the protein structure levels. Additionally, molecular interactions and functions of the NLRs pairs will be addressed. We will also exploit whole genome information of *M. oryzae* and rice to isolate novel pathogen effectors and their host target proteins.

**Expected Research Achievements and Scientific Significance**

By comparing the three AVR-NLR combinations, we will strive to unravel the shared mechanisms of AVR recognition by NLR. Identification of effector target proteins will facilitate the identification of novel rice susceptibility genes, which in turn allows us to develop blast resistant rice cultivars by introgression breeding. Successful accomplishment of the project should provide us with the fundamental understanding of organismal coevolution processes.

**Publications Relevant to the Project**


**Term of Project**

FY2015-2019

**Budget Allocation**

151,500 Thousand Yen

**Homepage Address and Other Contact Information**

http://genome-e.ibrc.or.jp/home
Title of Project: Molecular Basis of Infection Strategy in Plant Pathogenic Fungi: Host Recognition and Infection Structure Development

Yasuyuki Kubo
(Kyoto Prefectural University, Graduate School of Life and Environmental Sciences, Professor)

Research Project Number: 15H05780  Researcher Number: 80183797
Research Area: Plant Pathology
Keyword: Plant Pathogenic Fungi

Purpose and Background of the Research:

Colletotrichum species has specific trait as model plant pathogen for the study of infection mechanisms of plant pathogens. In our previous study, we have elucidated that plant pathogenic fungi recognize plant surface signals and form infection structure in response to environmental signals and that plant pathogenic fungi establish infection by communicating through interphase formed between fungal cells and plant cells and construct biotrophic interaction by suppressing plant immunity. This study deals with the molecular analysis of host recognition and infection structure formation of Colletotrichum orbiculare, an anthracnose disease fungus, thus establish the model for infection strategy of plant pathogenic fungi. Through this approach, we are aiming to explore the potential target metabolisms and genes in which involved for the development of novel antifungal reagents harmonious to environment, in view of developing innovative plant disease management.

Research Methods:

We have identified genes and metabolisms that are essential for proper infection related morphogenesis and pathogenesis of C. orbiculare, which include melanin biosynthesis, signal transduction pathway, peroxisome function, cell wall integrity, and effector protein secretion. In this study, based on our previous cutting edge findings about fungal pathogenesis, we will further deepen the biology of plant-microbe interactions. Through this study, we are aiming to get fundamental understandings for the control of plant disease. Especially in this study, we divided the infection process into pre-penetration stage and post-penetration stage. In the pre-penetration stage, we will try to understand the mechanisms, how plant pathogenic fungi recognize host plant for the initiation of infection, and in the post-penetration stage, we will focus on how fungal pathogens manage plant immunity from the view point of the functional mechanisms of secreted effector proteins.

Expected Research Achievements and Scientific Significance:

In this study, we will obtain essential data for the understanding of infection mechanisms of plant pathogenic fungi. The study will constitutes fundamentals for the development of novel and challenging way of plant disease control. Our study will also contribute basic biology in the field of plant-microbe interactions and developmental biology of filamentous fungi.

Publications Relevant to the Project:


Term of Project: FY2015-2019

Budget Allocation: 98,500 Thousand Yen

Homepage Address and Other Contact Information:
http://ykubo.blog.eonet.jp/
Title of Project: Analysis on Molecular Nutritional Functions of Bile Acids as a Feeding Signal, and Regulation of Metabolic Response to Feeding by Food Factors

Ryuichiro Sato
(The University of Tokyo, Graduate School of Agriculture and Life Sciences, Professor)

Research Project Number: 15H05781  Researcher Number: 50187259
Research Area: Agricultural Chemistry, Food Sciences
Keyword: Bile acids, Feeding signal, TGR5, FGF15/19

[Purpose and Background of the Research]
Physiological events that induce the most dramatic metabolic changes in humans are fasting and refeeding. Insulin secretion is induced by rise in blood glucose in response to feeding. It is convinced that insulin resistance caused by reduced sensitivity of insulin receptors associated with high serum insulin levels leads to the onset of multiple diseases including metabolic syndrome. Despite insulin resistance hepatic fatty acid and triglyceride synthesis induced by insulin action remains elevated, thereby causing fatty liver and lipid metabolism disorder. Recent studies have revealed that several types of signals delivered by bile acids secreted from the gallbladder in response to feeding stimulation function as regulatory factors to orchestrate metabolic responses to feeding like insulin does. These findings, therefore, imply that adjusting unhealthy metabolic responses to feeding which are caused by out-of-control insulin actions resulting from obesity or overeating, by bile acids is strongly desired. For this purpose the molecular basis of the feeding responsive pathway controlled by bile acids needs to be fully understood. The molecular basis will provide a new approach to improve metabolic disorders by adjusting metabolic responses to feeding through actions of food factors. The aim of this project is to elucidate the mechanism of bile acid-dependent metabolic response to feeding, and to show a new concept on bile acid functions and importance of the bile acid-dependent feeding signal pathway as a novel target for functional food factors.

[Research Methods]
The molecular basis on the relationship between alteration in metabolic responses to feeding and reduced feeding signals resulted from inhibition of bile acid uptake through its intestinal transporter is analyzed by multiple methods. Food factors that interact with bile acids or directly hinder the activity of their transporter are examined on the molecular mechanism for their mode of actions and regulation of metabolic responses to feeding. The regulatory mechanism of metabolic response to feeding through the bile acid receptor TGR5 in the small or large intestine and liver is investigated in vivo or in vitro. The mode of action of FGF15/19, a mediator of bile acid signals, as well as their receptor is examined at a molecular level. These studies will elucidate the molecular nutritional basis of ameliorating effects on metabolic response to feeding by reduced bile acid-dependent feeding signals, leading to novel food science researches from the perspective of exploring functional food factors with the capacity to exert ameliorating effect on metabolic response to feeding.

[Expected Research Achievements and Scientific Significance]
There is significant value to verify the new role of bile acids as a feeding signal so as to improve disorders of metabolic response to feeding. Findings confirmed in the current project are thought to lead to innovation by exploring functional food factors with the capacity to reduce metabolic responses to feeding.

[Publications Relevant to the Project]

[Term of Project] FY2015-2019

[Budget Allocation] 147,700 Thousand Yen

[Homepage Address and Other Contact Information]
http://webpark1213.sakura.ne.jp/aroytsato@mail.ecc.u-tokyo.ac.jp
Title of Project: Identification of Bull Pheromone and its Application to the Improvement of Fertility

Kei-ichiro Maeda
(The University of Tokyo, Graduate School of Agriculture and Life Sciences, Professor)

Research Project Number: 15H05782 Researcher Number: 30181580
Research Area: Animal Production Sciences
Keyword: Reproduction

Purpose and Background of the Research

Improvement of the fertility is one of the biggest concerns for Japan and other developed countries over the world. Fertility rate has been decreasing for many years despite the advancement of the science. The loss caused by the decreased fertility is around 100 billion Japanese Yen.

The current study aims to utilize the bull pheromone as a means to improve the fertility by stimulating reproductive axis. The ‘male effect’ was found decades ago in sheep and goats and the existence of a pheromone has been suggested by many experiments. In cows, the presence of bulls has also been suggested to improve the fertility. The release of gonadotropin-releasing hormone (GnRH)/gonadotropin, therefore, would be kept at a normal level by bull pheromone in cows. The absence of bull pheromone in a farm with the modern animal reproduction would cause a feeble estrus or reproductive disorders.

The present research focus on the isolation and identification of the bull pheromone to improve the fertility in milking and beef cows (Figure 1).

Figure 1 Conceptual illustration Left, pheromone released from the nose-ring is sensed by pheromone receptors to stimulate hypothalamic KNDy neurons and then GnRH/LH release resulting in ovarian estrogen secretion. Right, GnRH pulses are slowed-down in anaestrous cows and estrogen secretion is suppressed at a low level. The exposure to pheromones enhances the pulse frequency to stimulate estrogen secretion and then estrus and ovulation.

Research Methods

Our first attempt is to get bioassay systems to detect the pheromonal activity in bull samples. We will establish an immortalized bull vromeronasal cells and/or cell lines expressing every pheromone receptors in order to detect the pheromone activity in bull samples. In addition, we will take another way to identify pheromone activity with gonadotropin pulses and hypothalamic multiple unit activity of KNDy neurons.

Expected Research Achievements and Scientific Significance

The bull pheromone will be the second primer pheromone following the male goat pheromone. It would be of great scientific interests how these specific pheromones interact with each vromeronasal pheromone receptors.

From the application point of view, the development of pheromone preparation would be a novel method to improve the fertility of milking and beef cows.

Related Publications


Term of Project: FY2015-2019

Budget Allocation: 144,200 Thousand Yen

Homepage Address and Other Contact Information

akeimaed[at]mail.ecc.u-tokyo.ac.jp
Title of Project: Development of Novel Anti-Infectious Drugs Exhibiting Therapeutic Effects

Kazuhiyo Sekimizu
(The University of Tokyo, Graduate School of Pharmaceutical Sciences, Professor)

Research Project Number: 15H05783 Researcher Number: 90126095
Research Area: Environmental and hygienic pharmacy
Keyword: Microbiology and infectious diseases, Pathogenicity

### Purpose and Background of the Research
The development of novel anti-infectious drugs with therapeutic effects is urgently needed to establish effective strategies against multidrug-resistant pathogens. Current strategies, however, are inadequate and the number of newly discovered drugs has dramatically decreased, resulting in a very limited number of anti-infectious drugs with novel mechanisms. One possible reason for this is that the behavior of pathogenic bacteria in test tubes differs considerably from that in hosts. In this project, we will focus on gene products of pathogens that are necessary for pathogenesis in the host environment. To achieve this goal, we will identify the genes in pathogens necessary for pathogen proliferation and pathogenesis in host animals. Based on the findings, we will establish screening systems to identify inhibitors against the gene products and establish a method for developing antibacterial agents with novel mechanisms of action. Our project also aims to elucidate the molecular aspects of bacterial pathogenesis.

### Research Methods
1. Screening of pathogenic genes in bacteria using silkworms
We have established silkworm infectious disease models with human pathogenic bacteria. Using this model, we will identify deletion mutants of pathogenic bacteria whose pathogenesis is decreased compared with the wild-type strain. We will also identify bacterial genes whose expression is appreciably increased in mouse organs compared with that in test tubes. The decreased pathogenesis of the gene deletion mutants will be confirmed in mouse infection models.

2. Establishment of an assay system to screen novel antibacterial agents
We will then establish methods for biochemical analysis of the enzymes encoded by the pathogenic genes identified in this study. Using these methods, we aim to elucidate the functions of the enzymes. The assay systems we develop will then be used to screen for inhibitors.

### Expected Research Achievements and Scientific Significance
1. Understanding bacterial pathogenesis
Based on comprehensive analyses of data obtained in this project, we will identify novel genes responsible for bacterial pathogenesis. Biochemical analysis of the functions of the products of the responsible genes will allow us to uncover networks of gene expression involved in bacterial pathogenicity. These findings will contribute to our understanding of bacterial pathogenesis in the host.

2. Development of novel anti-infectious drugs
This project will identify novel genes necessary for bacteria to survive in the host environment. Evaluating the products of these novel genes will lead to potential targets for drug development. Bacterial growth inhibitors obtained by screening will be useful seed compounds for anti-infectious treatments.

### Publications Relevant to the Project


### Term of Project
FY2015-2019

### Budget Allocation
154,500 Thousand Yen

### Homepage Address and Other Contact Information
http://www.f.u-tokyo.ac.jp/~bisei/
Title of Project: Regulatory Mechanism of Immunoglobulin Gene Diversification and Genome Instability by RNA-Editing Catalyzed by Activation-Induced Cytidine Deaminase (AID)

Tasuku Honjo
(Kyoto University, Graduate School of Medicine, Guest Professor)

Research Project Number: 15H05784  Researcher Number: 80090504
Research Area: Medicine, Dentistry and Pharmacy
Keyword: DNA breaks, Recombination, Acquired immunity, Immunological memory

Purpose and Background of the Research
AID is the central and critical enzyme in generation of immunoglobulin (Ig) diversity and immunological memory which determines the efficacy of vaccination. AID employs Topoisomerase 1 (Top1) for DNA cleavage in class switch recombination (CSR) and somatic hypermutation (SHM) and induces genomic instability by causing aberrant DNA cleavage. This project aims to clarify the mechanism for Top1 translation regulation by RNA editing of the putative miRNA precursor(s) with AID and its cofactor hnRNP K. The target specificity of Top1 should be also elucidated. In addition we will identify the mRNA edited by AID and hnRNP L and AID will identify the proteins in the cleavage site-specific complex. The analysis of miRNAs binding to the complex of hnRNP L and AID will identify the proteins essential for DNA repair and recombination after DNA breaks. The function of these novel proteins will be associated with Brd4 which makes synopsis between distant two DNA break loci. Top1 covalently bound to the 3' end of DNA should be processed for efficient repair. The resection mechanism of Top1 from 3' end of DNA will be elucidated.

Expected Research Achievements and Scientific Significance
The elucidated mechanism of acquired immunity will be applied for the strategy of vaccine development. This project will clarify the function of hnRNP family proteins, which are largely unknown so far and contribute to the understanding of tumorigenesis by AID. This project will give the clear-cut view for pathogenesis of transcription-coupled genomic instability caused by Top1 deregulation, such as neuronal diseases and cancer. Identification of hypomorphic mutations of Top1, hnRNP K and hnRNP L in human will allow to assess a risk for polygenic diseases such as cancer or immunodeficiency.

Research Methods
The miRNA precursor(s) will be isolated by serial immunoprecipitation (IP) with AID and hnRNP K. Sequencing of these precursor(s) will unravel the exact base position edited by AID. The genome-wide analysis of non-B DNA structure by psoralen binding method and ChIP seq analysis detecting the accumulation of H3K4me3, Top1 and FACT complex will reveal the mechanism specifying the targets of AID-induced DNA breaks. iChIP methods using the cell line which has the lexA binding sequence in the S region will reveal proteins in the cleavage site-specific complex. The analysis of miRNAs binding to the complex of hnRNP L and AID will identify the proteins essential for DNA repair and recombination after DNA breaks. The function of these novel proteins will be associated with Brd4 which makes synopsis between distant two DNA break loci. Top1 covalently bound to the 3' end of DNA should be processed for efficient repair. The resection mechanism of Top1 from 3' end of DNA will be elucidated.

Publications Relevant to the Project

Term of Project: FY2015-2018
Budget Allocation: 153,500 Thousand Yen

Homepage Address and Other Contact Information
http://www2.mfour.med.kyoto-u.ac.jp/
Title of Project: Engulfment of Apoptotic Cells and Asymmetry of Plasma Membranes

Shigekazu Nagata
(Osaka University, Immunology Frontier Research Center, Professor)

Research Project Number: 15H05785  Researcher Number: 70114428
Research Area: Biochemistry, Molecular biology
Keyword: Apoptosis, Macrophage, Phosphatidylserine, Flippase, Scramblase

[Purpose and Background of the Research]
Macrophages engulf apoptotic cells using Tim-4, MFG-E8 and Mer/Protein S that recognize phosphatidylserine (PtdSer). If this process does not occur smoothly, it will cause autoimmune disease. Plasma membranes consist of two layers. In healthy cells, PtdSer is exclusively localized in the inner leaflets, but exposed in apoptotic cells. ATP11C work as a flippase that translocates PtdSer from outer to inner leaflets, while Xkr8 scrambles PtdSer during apoptosis.

In this project, we will perform the following studies. (1) Interaction of Mer and its homologues (Tyro3 and Axl) with their ligands (Protein S and Gas 6), and their ability to engulf apoptotic cells. (2) Effect of Tim-4 on MerTK kinase activity, and identification of Mer’s targets. (3) Physiological functions of Xkr4 and Xkr9 that work as a scramblase. (4) Tissue distribution of other P4-type ATPases and their function as a flippase.

[Expected Research Achievements and Scientific Significance]
This project is fully based on our own previous results. When this project is accomplished, it will reveal the molecular mechanism and physiological role of the engulfment of apoptotic cells. Our studies on flippases and scramblases in this project will elucidate how the asymmetrical distribution of phospholipids is maintained in healthy cells. It will also reveal why and how the asymmetry of plasma membrane is broken in some physiological settings. Our previous study on the molecules that recognize PtdSer indicated that if apoptotic cells are not properly engulfed by macrophages, it will cause autoimmune diseases. The de-regulation of scramblase and flippase may also cause various diseases, and the outcome of this project would contribute to our understanding of human diseases such as autoimmune diseases.

[Publications Relevant to the Project]

[Research Methods]
(1) By establishing cell lines that express only Mer, Axl, or Tyro3 in the absence or presence of Tim-4, we will study their ability to engulf apoptotic cells. We prepare the extracellular region of Mer, Axl, and Tyro3, and biochemically study their interaction with Protein S or Gas6. (2) Using the immunoprecipitation followed by Western blotting, we will study the association of Tim-4 and Mer. (3) By establishing knock-out mice, we will try to elucidate the physiological function of Xkr4 and Xkr8. (4) By expressing each member of the P4-ATPase family in ATP11C-null cells, we will study whether P4-ATPases other than ATP11C have flippase activity or not.

[Term of Project] FY2015-2019

[Budget Allocation] 118,100 Thousand Yen

[Homepage Address and Other Contact Information]
http://biochemi.ifrec.osaka-u.ac.jp/
snagata@ifrec.osaka-u.ac.jp
Title of Project: Previously Unappreciated Roles for Basophils in Health and Disease

Hajime Karasuyama
(Tokyo Medical and Dental University, Graduate School of Medical and Dental Sciences, Professor)

Research Project Number: 15H05786 Researcher Number: 60195013
Research Area: Experimental pathology, Immunology
Keyword: Inflammation, Allergy and immune-related disorders, Infection, Animal models

【Purpose and Background of the Research】
Basophils are the least common granulocytes and share some features with tissue-resident mast cells. Therefore, they have often erroneously been considered as precursors or blood-circulating subsets of mast cells, and their functional significance remained uncertain for a long time. We have recently developed novel tools for functional analysis of basophils, and demonstrated that basophils play important roles, distinct from those by mast cells, in parasitic infections, allergic and inflammatory responses. However, molecular mechanisms by which basophils elicit reactions remain to be determined. In this study, by using novel analytical tools, we clarify mechanisms underlying migration and activation of basophils as well as effector molecules produced by basophils under physiological and pathological conditions, and seek possible application of our findings to the treatment of inflammatory and infectious diseases.

【Research Methods】
1. Elucidation of mechanisms by which basophils elicit and regulate inflammation: We identify pro- and anti-inflammatory mediators derived from basophils and their target cells and molecules.
2. Elucidation of mechanisms by which basophils contribute to protection against parasitic infections: We examine which molecules produced by basophils are involved in parasite expulsion and how they work.
3. Elucidation of mechanisms underlying basophil migration to and accumulation in peripheral tissues: We examine what triggers basophil migration from peripheral blood into tissues.
4. Elucidation of mechanisms underlying basophil activation: We seek a ligand(s) of CD200R3, an activating receptor selectively expressed by basophils and mast cells, and examine the activation pathway through this receptor.

【Expected Research Achievements and Scientific Significance】
As many as one-thirds of people living in developed countries, including Japan, suffer from allergic disorders whereas many parasitic infections are still dominant in tropical areas. We hope that our study on basophils helps develop new drugs for allergy treatment and vaccines against parasitic infections.

【Publications Relevant to the Project】

【Term of Project】FY2015-2018

【Budget Allocation】154,000 Thousand Yen

【Homepage Address and Other Contact Information】
Title of Project: Elucidation of the Host’s Homeostatic Responses by the Regulation of Immune System and its Application to the Prevention and Treatment of Immunological Disorders

Tadatsugu Taniguchi
(The University of Tokyo, Institute of Industrial Science, Project Professor)

Research Project Number: 15H05787  Researcher Number: 50133616
Research Area: Molecular Immunology, Infection, Cancer, Inflammation
Keyword: Immune signaling, Homeostasis, Innate Immunity, Inflammation

Purpose and Background of the Research

The objective of this research project is to elucidate the underlying molecular mechanisms by which the immune system regulates homeostasis. Immune dysfunction is a critical factor underpinning autoimmune, cancer, and infectious diseases and this project will facilitate the basis for disease prevention and treatment.

Immune system activation in response to invading pathogens, mediated by recognition of pathogen-derived molecules called PAMPs, is critical for pathogen clearance and maintenance of host homeostasis. On the other hands, recent studies have revealed that the immune system also recognizes host-derived molecules, typically released by dead cells, termed DAMPs which have gained much attention for their involvement in various diseases (Rubartelli A. Trends Immunol., 28: 429-436, 2007). Yet, the nature for how DAMPs activate immune responses is not well characterized. As such, little is known about how these DAMP-mediated immune responses contribute to the regulation of host homeostasis.

Research Methods

Recent data from our laboratory show that DAMPs and analogue molecules activate immune responses to modulate homeostasis of the host. In particular, we have developed chemical compounds and decoy oligonucleotides which target putative DAMP or DAMP receptors and related molecules. On the basis of these data, we focus on the following four research projects listed below.

1. Clarify the regulatory mechanisms of inflammation and immune responses by dead cell-derived molecules.
2. Identify molecules from living host cells recognized by innate receptors that contribute to the maintenance of the host homeostasis.
3. Describe the role of molecules derived from gut microbiota and their interaction with host molecules for the maintenance of homeostasis.
4. Identify the targets of host-derived molecules utilizing by newly discovered chemical compounds, and clarify the immune regulatory mechanisms mediated by these compounds.

Expected Research Achievements and Scientific Significance

This study will provide new insight into the basic concept of how to modulate immune responses to keep the host’s homeostatic responses by self-derived molecules. Expanding on our preliminary, yet advanced research and utilizing our low molecular weight compounds and decoy oligonucleotide will enable us to modulate the immune system in novel ways. The anticipated findings will contribute to our understanding of the pathogenesis of autoimmune, cancer and infectious diseases; and may spawn new concepts and avenues for the treatment of diseases associated with immunity and inflammation.

Publications Relevant to the Project


Term of Project: FY2015-2019

Budget Allocation: 132,300 Thousand Yen

Homepage Address and Other Contact Information:
http://www.iis.u-tokyo.ac.jp/~mol-immu/
Title of Project: Development of a Novel Strategy for Life Style Disease through Exploration of the Roles of Mineral- and Gluco-Corticoids in Hypertension and Organ Dysfunction

Toshiro Fujita
(The University of Tokyo, Research Center for Advanced Science and Technology, Emeritus Professor/Project Researcher)

Research Project Number: 15H05788  Researcher Number: 10114125
Research Area: Medicine, Dentistry, and Pharmacy
Keyword: Nephrology, Hypertension

[Purpose and Background of the Research]
We have been studying about the organ dysfunction related to life style disease from many aspects. Excess of adrenal hormones, aldosterone and cortisol, induces hypertension and kidney injury. We clarified that activation of the receptors of these hormones plays a key role in hypertension and organ dysfunction, independent of serum concentrations of the hormones. We showed that a small GTP binding protein Rac1 (1) and decrease of histone deacetylase 8 (2) induces hypertension by activating MR and GR, respectively.

We will explore the mechanisms underlying activation of MR and GR by investigating 3 points described below and aim to develop a novel strategy for treatment of hypertension and kidney injury. 1) Exploration of the role of Rac1-MR pathway in the organ dysfunction. Activation of Rac1- MR pathway in the kidney induces glomerular damage and hypertension. We will clarify the respective sites of Rac1 activation involved in glomerular damage and hypertension. We also investigate the roles of Rac1-MR pathway in cardiac dysfunction. 2) Exploration of novel targets of MR and GR pathways. Using kidney-specific MR and GR knockout mice, we will identify the novel disease modifying genes regulated by MR and GR. 3) We also clarify the mechanisms underlying MR and GR activation by analyzing receptor modifications and epigenetic changes of the promoter regions of genes newly identified in 2).

[Research Methods]
Site-specific (glomerular and tubular) knockout mice will be analyzed for determining the role of Rac1-MR pathway of the kidney. Cardiomyocyte-specific Rac1 and MR knockout mice will also be analyzed. The role of pendrin in hypertension will be determined by genetically modified mice.

Novel target genes of MR and GR will be clarified by analyzing site-specific MR and GR knockout mice. Furthermore, novel switch mechanisms for MR and GR will be discovered by analyzing the promoter modifications by MR, GR, and epigenetic states of the novel target genes using techniques including ChIP sequencing. Finally, the findings obtained from mice will be proved by using human kidney biopsy samples.

[Expected Research Achievements and Scientific Significance]
The novel genes regulated by MR and GR and mechanisms underlying MR and GR activation identified in the present study are expected to be the targets for novel therapy against hypertension and organ dysfunction. Exploration of the mechanisms underlying aberrant epigenetic changes would pave the way for the development of novel means for prevention and/or reversal of pathologic states considered to be irreversible.

[Publications Relevant to the Project]

[Term of Project] FY2015-2019

[Budget Allocation] 153,800 Thousand Yen

[Homepage Address and Other Contact Information]
http://www.c-epi.rcast.u-tokyo.ac.jp/index.html
Title of Project: Development of a Novel Anti-Aging Strategy by Elucidating the Mechanisms Regulating Aging through a Muscle Centric Organ Network

Kohjiro Ueki  
(The University of Tokyo, Graduate School of Medicine, Professor)

Research Project Number: 15H05789  Researcher Number: 00396714
Research Area: Diabetes and Metabolism
Keyword: Aging, Signal Transduction, Diabetes

Purpose and Background of the Research
Japan, which has been rapidly becoming an aged society, urgently seeks for an efficient strategy to provide the people with a healthy and socially active long lifespan. Compared to the Caucasians, the East Asians are prone to develop a life-style related disease, such as diabetes and cardiovascular disease, by aging even in the non-obese or mildly obese state. Indeed, aging deteriorates muscle volume and quality, namely sarcopenia, leading to the development of insulin resistance and decline in activity of daily life. Sarcopenia impairs socially active and independent living of the aged people by causing a variety of life-style related diseases and frailty. We hypothesize that healthy muscle maintained by insulin signaling communicates with other organs thereby preventing whole body aging. The aim of this study is to elucidate the mechanisms of sarcopenia by impairment of insulin action and dysregulation of the muscle centric organ network, allowing us to develop a novel and efficient anti-aging therapy.

Research Methods
Based on the analyses of diabetic animal models, we hypothesized that aging impairs insulin signaling, especially Akt activity, in muscle, leading to the development of sarcopenia, which in turn exacerbates insulin resistance, resulting in further progress in sarcopenia. To assess this hypothesis, we have generated muscle specific Akt1/Akt2 double knockout (mAktDKO) mouse, and found that mAktDKO mice exhibit premature sarcopenia and whole body aging phenotypes, such as osteopenia. In this study, we try to identify pathways or factors downstream of Akt regulating sarcopenia and whole body aging by performing transcriptome, metabolome and signaling studies using mAktDKO mice and analyzing those mice with deletion of TSC2 or FoxO proteins. Furthermore, we try to explore the mechanism maintaining youth and homeostasis by investigating aging biomarkers in various tissues and cognitive functions of wild type and mAktDKO mice. Moreover, we will test the effect of specific nutrients or exercise on the prevention of sarcopenia in wild type and mAktDKO mice.

Expected Research Achievements and Scientific Significance
Through this project, useful biomarkers and compounds for preventing sarcopenia and aging will be developed. A novel disease concept, “Metabolocomotive syndrome”, caused by dysregulation of the muscle centric organ network, will be established.

Strategies for preventing “Metabolocomotive Syndrome” and Aging

Research (S)

Publications Relevant to the Project
Iwabu M et al. Adiponectin and AdipoR1 regulate PGC-1alpha and mitochondria by Ca(2+) and AMPK/SIRT1. *Nature* 464:1313-1319, 2010

Term of Project
FY2015-2019

Budget Allocation
153,800 Thousand Yen

Homepage Address and Other Contact Information
http://dm.umin.jp/dmsd/
In the 1980s, Streilein et al. introduced the term “skin-associated lymphoid tissue (SALT)” based on observations that revealed the existence of T cells and dendritic cells (DCs) in the skin and that T cells are activated in the skin draining lymph nodes. However, it remains unclear whether and how T cells are activated in the skin in the viewpoint of SALT. Therefore, SALT has remained conceptual.

Through the detailed examination of a skin specimen obtained from a patient with contact dermatitis, we discovered that dermal DCs (dDCs) form clusters with T cells just beneath the epidermal spongiosis. This observation suggests that dDC clustering plays a role in the elicitation phase of contact dermatitis.

In this context, we hypothesized that dDC clustering in contact dermatitis might be essential for memory T cell activation in the skin to elicit their acquired immune functions in the perspective of SALT.

Using two-photon microscopy, we discovered that dDCs are attracted by perivascular macrophages at the post-capillary venules in the elicitation phase of contact hypersensitivity (CHS), a murine model of contact dermatitis (Figure).

This sequential leukocyte cluster formation is essential for efficient activation of memory T cells; now it can be assumed as a lymphoid tissue. The structure does not exist in the steady state, but emerges in response to local inflammatory conditions. Herein, we propose this structure to be termed as “inducible SALT (iSALT)”. However, the role of iSALT remains to be clarified.

Firstly, we will examine whether iSALT enables naïve T cell priming or B cell class switching and antibody production in situ. Secondly, the functional and pathological differences between iSALT and other skin lymphoid structures will be determined. Thirdly, we will investigate whether iSALT or iSALT-like structures are established in other settings, such as atopic dermatitis and psoriasis.

To understand the role of iSALT will reveal the mechanism how the skin responds to the external stimuli, which will lead to the understanding of skin immune diseases. In addition, controlling the functions of iSALT will improve the efficacy of cancer vaccination via the skin.


[Term of Project] FY2015-2019

[Budget Allocation] 147,000 Thousand Yen

[Homepage Address and Other Contact Information] http://www.kuhp.kyoto-u.ac.jp/~skin/index.html
Title of Project: Achievement of Highly Accurate Diagnosis of Early Pancreatic Cancer in Japanese Patients through a Comprehensive/Integrated Approach

Masaki Mori
(Osaka University, Graduate School of Medicine, Professor)

Research Project Number: 15H05791  Researcher Number: 70190999
Research Area: Medical, Dental, and Pharmaceutical

Keywords: Pancreatic surgery

【Purpose and Background of the Research】
Gastrointestinal cancers account for 65% of the cancer deaths in Japanese patients, and pancreatic cancer is the most refractory among gastrointestinal cancers. However, its 5-year survival rate is 69% if it is diagnosed in the early stage of the disease. As is clear from these data, early diagnosis is extremely important in improving the treatment outcome in patients with pancreatic cancer, thus making early diagnosis through the development of novel biomarkers and appropriate medical interventions critical. In practical situations, however, conducting studies in patients with early pancreatic cancer is challenging because the number of patients is extremely small. Therefore, cases with clinically nonmetastatic early stage pancreatic cancer are strategically and systematically collected to analyze their data with high accuracy in the All-Japan System.

【Research Methods】
To develop novel biomarkers for the highly accurate diagnosis of early pancreatic cancer with sensitivity and specificity exceeding those of the current medical technology, it is important to collect Japanese samples (tumor [T] factor = blood/saliva [and tumor whenever possible] from patients with early pancreatic cancer) across the nation within a given time frame as much as possible and to obtain a complete, integrated understanding by examining the (T) factor together with environmental (E) factors and genetic background (P) as the trinity. We aim to elucidate the individual causal association over 5 years and to apply these results to develop a system for intellectual property maintenance, improve industrial infrastructure, widely conduct awareness programs among citizens, and deliver medical services.

【Expected Research Achievements and Scientific Significance】
(1) Pancreatic cancer often has a poor prognosis; thus, overcoming this disease can be a persistent desire of citizens. Early diagnosis is extremely important. (2) Early pancreatic cancer has been difficult to study. Establishment of a strategic research organization supported by the All-Japan System is essential to promote comprehensive development projects for problem-solving in order to achieve an early diagnosis. (3) This study is a strategic analysis specialized for the diagnosis of early pancreatic cancer based on a previous triune fusion-type study on colorectal and esophageal cancers. The (P) factor is expected to reduce development cost, while high quality is maintained by adding the (E) factor after confirming/utilizing information from genome-wide association studies. A triune fusion-type study (P + E + T) is conducted by collecting samples from patients with early pancreatic cancer (T factor) across the country. (4) This will be the first ever study in the world to conduct research on early pancreatic cancer by considering 3 factors in combination. (5) The importance of miRNA/exosomes in peripheral blood and cancer metabolites has received interdisciplinary attention.

Fundamental information that will help identify patients in the high-risk group (P and E factors), avoid high-risk habits (P and E factors), early diagnosis when examining pancreatitis or other diseases (P, E, and T factors), and create awareness programs for citizens for their appropriate understanding of the disease by providing consultations on inheritance to family members will be obtained. Thereby, a more accurate treatment strategy than the current one can be established.

【Publications Relevant to the Project】

【Term of Project】 FY2015-FY2019

【Budget Allocation】 JPY 153,800 Thousand Yen

【Homepage Address and Other Contact Information】
http://www.med.osaka-u.ac.jp/pub/gesurg/
**Title of Project:** Development of Innovative Treatment Targeting the Sphere Formation Mechanism Involving Cancer Stem Cells

Yoshihiko Maehara
(Kyushu University, Faculty of Medical Sciences, Professor)

**Research Project Number:** 15H05792  **Researcher Number:** 80165662
**Research Area:** Medicine, Dentistry and Pharmaceutical Sciences
**Keyword:** Cancer, Surgery, Cell/Tissue, Drug Responsiveness, Translational Research

### Purpose and Background of the Research

Recurrence of cancer is explainable if we assume the existence of cancer stem cells (CSCs) that are resistant to various therapies. One of the typical CSC research methods is the sphere formation assay, however, the specific molecular mechanism of cancer cell transformation resulting from sphere formation is unknown. In this research project, we will advance and accelerate sphere biology to elucidate the mechanism of sphere formation that makes cancer an intractable disease, with the aim of its therapeutic application (Figure 1).

![Sphere formation and expression of CXCR4](image)

- **GFP**
- **CXCR4**
- **Merge**

CXCR4 was detected by immunohistochemistry.

### Research Methods

1. The molecular biological mechanism of sphere formation will be elucidated. A molecule essential for sphere formation will be identified using a comprehensive method based on transcriptome, proteome, and metabolome analyses. A comprehensive search for inhibitors of sphere formation will be carried out using chemical libraries.
2. The relationship between changes after sphere formation and cancer stem cells will be elucidated. Downstream signals, genetic and epigenetic changes, and gene expression following sphere formation will also be analyzed.
3. Analysis will be performed using clinical samples. At our department and the institutions of co-collaborators, malignant tumor samples from tumor organs are systematically stored. Using these samples, expression analysis of the target molecule(s) identified through the above-mentioned research will be performed.
4. An innovative treatment modality for the inhibition of sphere formation will be developed.

### Expected Research Achievements and Scientific Significance

This research is unique in that its achievements can be applied both directly and indirectly to the fields of cancer stem cell research and regenerative medicine through an understanding of the new concept of “sphere biology” from a molecular biological perspective. In this research project, clarification of the relationship between sphere formation and cancer stem cells, identification of cancer stem cell niches, and analysis of these molecular biological characteristics will allow for the identification of therapeutic target molecule(s) to zero in on cancer stem cells and destroy them. In addition, the combination of such molecule(s) with existing treatments can be a potential breakthrough in cancer treatment.

### Publications Relevant to the Project


### Term of Project

FY2015-2019

### Budget Allocation

144,000 Thousand Yen

### Homepage Address and Other Contact Information

http://www.kyudai2geka.com