Title of Project : How sexual experience modulates innate behavior: a neurogenetic study in *Drosophila*

Daisuke Yamamoto
(Tohoku University, Graduate School of Life Sciences, Professor)

Research Project Number : 16H06371 Researcher Number : 50318812
Research Area : Behavioral genetics
Keyword : Courtship behavior

【Purpose and Background of the Research】

The mechanism whereby genes and the environment interact to shape a behavior remains an enigma despite its tremendous importance in understanding human nature. Molecular cloning of the *fru* gene and neuroanatomical demonstration of sexual dimorphisms in *fru*-expressing neurons led to the notion that *fru* functions as a master regulator of the formation of courtship neural pathways, which operate as hard-wired circuitries to generate genetically determined courtship behavior. Thus genetic mutations in *fru* result in changes in the courtship target choice in these males. Recent studies have challenged this view, by showing that inappropriate courtship is suppressed in *fru* mutants that are raised in isolation. The present proposal aims to determine the mechanism by which social experience affects the mate preference in *fru* mutants, thereby unraveling the molecular underpinning of gene-environment interactions in shaping the behavior.

【Research Methods】

The tethered male preparation is used to induce courtship behavior with photoactivation of brain neurons via channelrhodopsin. In vivo patch clamp is applied to *fru*-expressing single neurons to record plastic changes induced by social experience. The polymeraseII occupancy is determined at identified Fru-target genes by the TaDa method to detect possible changes associated with social experience.

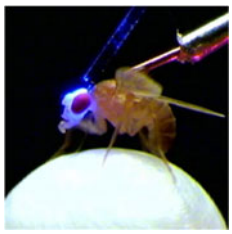
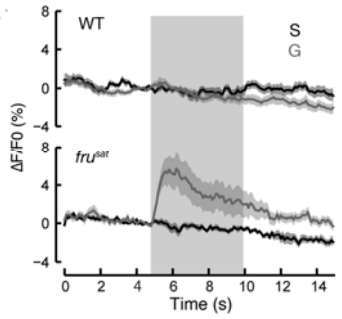


Figure 1 Courtship induced in a tethered male via channelrhodopsin-acitivation.



S: single housed
G: group housed
WT: wild type
fru^{sat}: *fru* mutant
Shadings: visual stimulation

Figure 2 Social effects on neural activities.

【Expected Research Achievements and Scientific Significance】

Although the importance of nature-nurture interplay in specifying complex behavioral traits has been recognized, its mechanistic bases remain obscure. This study will clarify the molecular and cellular mechanisms underlying the nature-nurture interaction in shaping behavior. It will not only renovate our view of the relation of mind and brain but also potentially yield a means to improve mental health.

【Publications Relevant to the Project】

・Kohatsu, S. and Yamamoto, D. (2015) Visually induced initiation of *Drosophila* innate courtship-like following pursuit is mediated by central excitatory state. Nat. Commun. 6, 6457.

【Term of Project】 FY2016-2020

【Budget Allocation】 140,900 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.biology.tohoku.ac.jp/lab-www/yamamoto_lab/

Title of Project : Integrated studies of regulation of neuronal function and development by kinesin superfamily motors, KIFs

Nobutaka Hirokawa
(The University of Tokyo, Graduate School of Medicine, Project Professor)

Research Project Number : 16H06372 Researcher Number : 20010085
Research Area : Molecular Cell Biology, Neuroscience, Developmental Biology
Keyword : Kinesin Motors, Microtubules, Neuroscience, Development

【Purpose and Background of the Research】

The intracellular transport is fundamental for cells by transporting various kinds of cargoes in cells in general. Kinesin superfamily proteins, KIFs are major players in this mechanism. Our molecular genetics also uncovered the mechanisms of important physiological processes such as higher brain function (**Neuron** 2010, 2011,2012), brain wiring(**Cell** 2003), left/right determination of our body, suppression of tumorigenesis, and control of nervous system development. The defects of these KIFs cause diseases such as neuropathy, brain malformation, brain tumor, laterality defect and megacolon (**JCB** 1998; **Cell** 1998; **Cell** 2001; **Cell** 2003; **Cell** 2009), epilepsy (**Neuron** 2012b), anxiety neurosis (**Cell Rep** 2013), hydrocephalus and female infertility (**Dev Cell** 2012), and Diabetes (**Dev Cell** 2014). Some KIFs have new functions such as KIF4 (**Cell** 2006) and KIF26A (**Cell** 2009) as signaling molecules and KIF2A (**Cell** 2003) and KIF19A as microtubule depolymerizers (**Dev Cell** 2012). However, there are numbers of KIFs whose functions are still unknown and the mechanisms of regulation of KIFs especially by phosphorylation for controlling motor activity and cargo binding are also largely unsolved. As for the neuroscience KIFs' involvement for higher brain function/neuronal activity/neuronal plasticity need to be solved and KIFs also could control fundamental mechanisms for development. Thus, there are numbers of problems which need to be solved and studies of them will contribute significantly not only for molecular cell biology /neuroscience/ developmental biology, but also for medical science.

【Research Methods】

Multidisciplinary approaches such as molecular cell biology, molecular genetics, biophysics and structural biology will be used.

【Expected Research Achievements and Scientific Significance】

In out project we will study following objects.
I) Analysis of the mechanism of regulation of KIFs: A) The mechanism of regulation of motility and transport of cargoes by phosphorylation of KIFs. We have developed a new quantitative method to analyze phosphorylation of KIFs and responsible kinases (**Cell Rep** 2015; **Neuron** 2015). We will focus on major KIF motors. B) The mechanism of depolymerization of microtubules by KIF2A and KIF19A.
II) The mechanism of regulation of neuronal plasticity, memory/learning, and neuronal functions by KIFs: A) a) Function of KIF21B for learning and memory and the mechanism of disorder caused by deletion of KIF21B. b) Role of KIF3 for neuronal plasticity and the mechanism of psychiatric disorder caused by defect of KIF3. c) Role of KIFs for development and neuronal plasticity of visual cortex at the critical period. d) Role of KIF17 on retrieval phase of learning and memory. B) a) Function of KIF1A for pain sensation and the mechanism of its defect caused by deletion of KIF1A. b) Function of KIF26 for pain sensation and the mechanism of hyper sensitivity for pain by deletion of KIF26.
III) The mechanism of regulation of development by KIFs: A) Function of KIF2A during brain development and the mechanism of malformation of cortical development and epilepsy caused by defect of KIF2A. B) New role of KIF3 for formation of morphogen gradient during development. These studies contribute significantly for molecular cell biology, neuroscience, developmental biology and medical science.

【Publications Relevant to the Project】

Hirokawa, N., et. al., Molecular motors in neurons: Transport mechanisms and roles in brain function, development, and disease. **Neuron** 68: 610-638, 2010.
Ichinose, S., et. al., Mechanism of activity-dependent cargo loading via the phosphorylation of KIF3A by PKA and CaMKIIa. **Neuron** 87: 1022–1035, 2015

【Term of Project】

FY2016-2018

【Budget Allocation】

142,900 Thousand Yen

【Homepage Address and Other Contact Information】

http://cb.m.u-tokyo.ac.jp

Title of Project : Mechanism and regulation of "Hit-and-Run" carcinogenesis by *Helicobacter pylori* CagA

Masanori Hatakeyama
(The University of Tokyo, Graduate School of Medicine, Professor)

Research Project Number : 16H06373 Researcher Number : 40189551
Research Area : Cancer Biology
Keyword : Carcinogenesis, Inflammation and cancer, Tumor microenvironment, Oncogene

【Purpose and Background of the Research】

Gastric cancer is the third-leading cause of cancer-related deaths, accounting for approximately 10% of total cancer deaths worldwide. Most if not all gastric cancer cases are etiologically associated with chronic *Helicobacter pylori* infection. In particular, the CagA oncoprotein of *H. pylori*, which is delivered into gastric epithelial cells, plays a key role in the development of gastric cancer by perturbing multiple intracellular signaling pathways. Once established, however, gastric cancer cells no longer require *H. pylori* or CagA for maintaining their malignant phenotypes, indicating that the neoplastic transformation of gastric epithelial cells follows a process of "Hit-and-Run" carcinogenesis. In this study, the process of gastric carcinogenesis will be investigated by dividing it into CagA-dependent and CagA-independent stages. Elucidation of the molecular mechanisms underlying each of these oncogenic stages will shed light on the mechanism of the "Hit-and-Run" carcinogenesis, which will pave the way for gastric cancer prevention.

【Research Methods】

Investigation of the CagA-dependent stage will focus on the role of CagA-SHP2 complex formation in determining gastric cancer risk through quantitative analysis, the physiological role and the oncogenic contribution of the newly identified SHP2 substrate parafibromin, the mechanism by which the level of CagA tyrosine phosphorylation is determined, and the pathophysiological collaboration of *H. pylori* CagA and EBV in the neoplastic transformation of gastric epithelial cells. The tyrosine-phosphorylated recombinant CagA protein will become a powerful tool that enables quantitative analysis for the study of gastric carcinogenesis. Investigation of the CagA-independent stage will be performed by establishing genetically engineered mice that conditionally switch on/switch off the expression of CagA. Genetic and epigenetic analyses of pre-neoplastic/neoplastic lesions induced by conditional CagA expression will uncover molecular mechanisms that confer CagA independence to epithelial cells.

【Expected Research Achievements and Scientific Significance】

This study will allow for a deeper understanding of the mechanism that mediates *H. pylori*-induced gastric cancer through comprehensive studies on "Hit-and-Run" carcinogenesis of the stomach by integrating multiple layers of investigation. Unique animal models to be established in this study should act as powerful experimental tools not only for the study of *H. pylori*-associated gastric cancer but also for searching for principles common to a variety of infection/inflammation-associated cancers. A molecular understanding of the mechanism underpinning the "Hit-and-Run" carcinogenesis of the stomach would enable prognosis prediction of individual *H. pylori* eradication in cancer prevention, thereby having important clinical significance in terms of precision medicine.

【Publications Relevant to the Project】

・Saju P, Murata-Kamiya N, Hayashi T, Senda Y, Nagase L, Noda S, Matsusaka K, Funata S, Kunita A, Urabe M, Seto Y, Fukayama M, Kaneda A, *Hatakeyama M. Host SHP1 phosphatase antagonizes *Helicobacter pylori* CagA and can be downregulated by Epstein-Barr Virus. **Nat Microbiol.** 1: 16026 (2016)
・Hatakeyama M. *Helicobacter pylori* CagA and gastric cancer: a paradigm for Hit-and-Run carcinogenesis. **Cell Host Microbe** 15: 306-316 (2014)

【Term of Project】 FY2016-2020

【Budget Allocation】 141,600 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.microbiol.m.u-tokyo.ac.jp

Title of Project : Investigation of the novel mechanisms underlying tumorigenesis due to aberrant Wnt signal networks

Akira Kikuchi
(Osaka University, Graduate School of Medicine, Professor)

Research Project Number : 16H06374 Researcher Number : 10204827
Research Area : Biological Sciences
Keyword : Wnt signaling, Arl4c, Dkk1, CKAP4, Wnt5a

【Purpose and Background of the Research】

Wnts are secretory proteins that are conserved evolutionally and regulate two intracellular signaling pathways: β-catenin-dependent and -independent. Both pathways are essential for animal development, whereas the roles of Wnt signaling in the post-natal life are not well understood.

Wnt signaling abnormalities are frequently observed in human cancers, and the extensive trials to develop new cancer therapeutics which target Wnt signaling molecules, especially the β-catenin-dependent pathway components, have been long continued. The good outcomes, however, have not been obtained yet. Evidence has accumulated that the β-catenin-independent pathway is also involved in tumorigenesis, but the significance is not fully understood because the activation of Wnt5a/β-catenin-independent signaling promotes or suppresses tumorigenesis in a cancer cell context.

In this study we aim to clarify unresolved issues in the Wnt signaling field. Especially the mechanisms by which novel downstream signaling of the β-catenin pathway causes tumor formation and those by which the β-catenin-independent pathway controls tumorigenesis and inflammation will be clarified (Figure 1).

【Research Methods】

1. Regulation of expression and mode of action of Arl4c in tumorigenesis

We identified Arl4c as a new downstream molecule of the β-catenin -dependent pathway. How Arl4c is expressed and activated and how the expression causes tumorigenesis are examined.

2. Modes of activation and action of Dkk1-CKAP4 signaling in tumorigenesis

CKAP4 was identified as a novel receptor of Dkk1, a direct target of the β-catenin-dependent pathway. How Dkk1-CKAP4 signaling promotes tumorigenesis is investigated.

3. Regulation of expression and mode of action of Wnt5a in tumorigenesis with inflammation

How Wnt5a is expressed in fibroblasts and cancer cells by inflammatory cues and how cancer cells and immune cells are mutually interacted are examined.

【Expected Research Achievements and Scientific Significance】

The following mechanisms would be clarified.

1. The molecular mechanism by which the novel signaling downstream of the β-catenin-dependent pathway causes tumor formation.
2. The molecular mechanism by which the Wnt5a/ β-catenin-independent pathway causes tumor formation and inflammation.

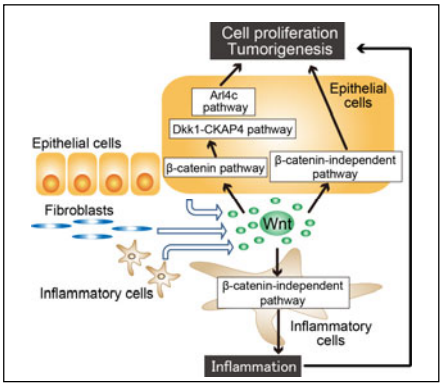


Figure 1

【Publications Relevant to the Project】

・Kimura, H., Fumoto, K., Shojima, K., Nojima, S., Osugi, Y., Tomihara, H., Eguchi, H., Shintani, Y., Endo, E., Inoue, M., Doki, Y., Okumura, M., Morii, E., and Kikuchi, A. CKAP4 is a Dickkopf1 receptor and is involved in tumor progression. J. Clin. Invest. doi:10.1172/JCI84658, 2016
・Matsumoto, S., Fujii, S., Sato, A., Ibuka, S., Kagawa, Y., Ishii, M., and Kikuchi, A. A combination of Wnt and growth factor signaling induces Arl4c expression to form epithelial tubular structures. EMBO J. 33, 702-718, 2014

【Term of Project】 FY2016-2020

【Budget Allocation】 136,300 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.med.osaka-u.ac.jp/pub/molbiobc/
akikuchi@molbiobc.med.osaka-u.ac.jp

Title of Project : Molecular mechanism and physiological understanding of Autophagy

Yoshinori Ohsumi
(Tokyo Institute of Technology, Frontier Research Center, Professor)

Research Project Number : 16H06375 Researcher Number : 30114416
Research Area : Molecular Cell Biology
Keyword : autophagy, proteolysis, RNA degradation, ATG, yeast

【Purpose and Background of the Research】

Autophagy is a fundamental degradative pathway that occurs within the cell's own lytic compartment (the vacuole or the lysosome) and is conserved throughout virtually all eukaryotic cells. A detailed description of autophagy is therefore indispensable for a complete understanding of the basic unit of life, the cell. Lending on the 27 years of experience of the leading researcher, this project will use yeast as a model organism to undertake a systematic and rigorous biochemical interrogation of the yet-uncharacterised physiological functions of autophagy, providing a comprehensive picture of the role of autophagy in the cell.

【Research Methods】

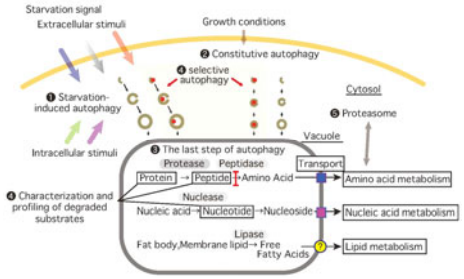
1. Understanding the conditions of autophagy induction. We aim to describing the induction of autophagy under a range of nutrient rich and deplete conditions, with a particular emphasis induction during starvation for zinc and iron and the physiological role of autophagy as a response to such conditions.

2. Uncovering the mechanism of autophagy induction during carbon-source starvation We have found that as part of their response to carbon deprivation, yeast induce autophagy when grown on non-fermentable sources of carbon. We will investigate the induction signals and identify molecular substrates of autophagy under these conditions.

3. Establishment of an analytical regime for autophagic proteolysis. We will generate strains of yeast lacking all nine amino-and carboxyl-peptidases of the vacuole, and investigate the phenotypic outcomes of this manipulation under starvation conditions. Using these mutants, autophagy-derived peptides that accumulate in the vacuole will be assessed by biochemical and cell biology analytical techniques. In addition, we will identify proteins degraded by autophagy under a range of conditions using mass spectrometric analyses of these peptides. The establishment of this analytical regime is therefore also a priority in our work.

4. Examination of the degradation of RNA by autophagy We will examine vacuolar degradation of RNA and elucidate enzymes connected to nucleotide metabolism in the cell. This involves the establishment of a comprehensive experimental regime that is able to identify specific substrates of autophagic RNA degradation. At the outset, we will examine the degradation of rRNA, tRNA, ncRNA and others, before examining the meaning of this degradation. In this project, we also aim to develop highly-sensitive assays for the detection of modified bases and nucleotides released from the cell, enhancing the potential for autophagy as a quantitative indicator of cellular processes.

5. Other lines of enquiry. We will also undertake analyses of the mechanism of autophagic secretion, the mechanistic link between autophagy and the initiation and arrest of cell growth, constitutive autophagy and other projects related to the physiological role of autophagy in the cell.



【Expected Research Achievements and Scientific Significance】

Autophagy is currently garnering the most attention of the diverse fields of cell biology. Divergent physiological roles of autophagy have been uncovered, but their explicit implications remain unclear. One reason why is because biochemical analysis of the lysosome is difficult. Using the unique features of the yeast vacuole, we aim to address the important questions of what, when and how autophagy degrades cellular components in the cell, which are key to the continued development of this important field and our understanding of cell biology.

【Publications Relevant to the Project】

1.Takeshige, K., and, Ohsumi Y et.al Autophagy in yeast demonstrated with proteins-deficient mutants and its conditions for induction. J. Cell Biol., 119, 301-311 (1992)
2.Tsukada, M., and Ohsumi, Y. Isolation and characterization of autophagy-defective mutants of Saccharomyces cerevisiae. FEBS Lett., 333, 169-174 (1993)
3.Mizushima, N., and Ohsumi, Y.et. al A protein conjugation system essential for autophagy. Nature, 395, 395-398 (1998)
4.Nakatogawa, H. and Ohsumi Y et.al Atg8, a ubiquitin-like protein required for autophagosome formation, mediates membrane tethering and hemifusion. Cell, 130, 165-178(2007)
5.Huang H*, Kawamata T*, Ohsumi Y**, Fukusaki E**et.al. Bulk RNA degradation by nitrogen starvation-induced autophagy in yeast. EMBO J. 34, 154-168 (2015)

【Term of Project】FY2016-2020

【Budget Allocation】143,700 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.ohsumilab.aro.iri.titech.ac.jp/
yohsumi@iri.titech.ac.jp

Title of Project : Evoking limb regeneration from non-regenerative animals

Kiyokazu Agata
(Gakushuin University, Faculty of Science, Professor)

Research Project Number : 16H06376 Researcher Number : 70167831
Research Area : Biology
Keyword : Development & Differentiation, Organogenesis, Regeneration, Epigenomics

【Purpose and Background of the Research】

We found a new principle of regeneration, called <distalization & intercalation> (ref.1). To restore lost portions of bodies after amputation, animals regenerate the most distal portion at first and then reorganize intermediate regions between the newly formed distal portion and remaining tissues by intercalation. Based on this new principle, we have succeeded in inducing dormant regenerative ability from non-regenerative animals by artificial manipulation (ref.2-4).

Thus, as s next target, we focus on limb regeneration. Newts can regenerate limbs after amputation. However, in the case of frogs, they lose limb regeneration ability after metamorphosis. It is known that frogs stop expressing the *Shh* gene after metamorphosis, and that a limb specific enhancer (MFCS1) may have an important role to form positive feedback between FGF and Shh for inducing intercalation. Therefore, in this study, we are trying to activate *Shh* expression in frogs even after metamorphosis by modulating the frog MFCS1 sequence.

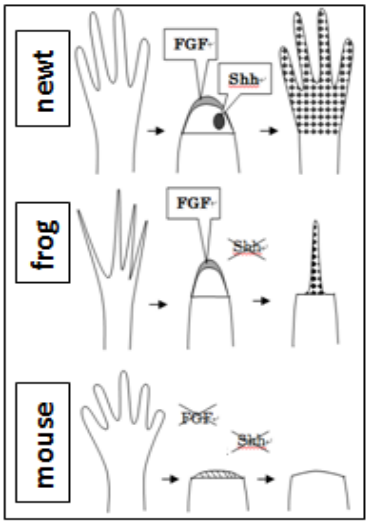


Fig. 1. Comparison of regenerative ability among newt, frog and mouse.

【Research Methods】

As the first step, we investigate the reasons why MFCS1 does not work after metamorphosis in frogs. A Tohoku University group suggested that MFCS1 is highly methylated after metamorphosis. So, we plan to replace the frog MFCS1 with newt MFCS1 by a genome editing technology, such as the CRISPR/Cas9 system, and vice versa. Through these analyses we will be able to elucidate the reason why frog MFCS1 is inactivated after metamorphosis. We will also analyze how MFCS1 functions to form the FGF/Shh positive feedback loop. Based on these analyses, we will also attempt to induce limb regeneration in mouse limbs.

【Expected Research Achievements and Scientific Significance】

When we succeed in inducing limb regeneration from frog and mouse, we will be able to provide a new strategy for regenerative medicine.

【Publications Relevant to the Project】

1. Unifying principles of regeneration I: epimorphosis versus morphallaxis.
K. Agata, Y. Saito and E. Nakajima
Dev. Growth Differ., 49, 73-78 (2007)
2. The molecular logic for planarian regeneration along the anterior-posterior axis
Y. Umesono, J. Tasaki, S. Yazawa, K. Itomi, O. Nishimura, Y. Tabata, F. Son, N. Suzuki, R. Araki, M. Abe and K. Agata
Nature, 500, 73-76 (2013)
3. Reintegration of the regenerated and the remaining tissues during joint regeneration in newts, *Cynops pyrrhogaster*.
R. Tsutsumi, T. Inoue, S. Yamada and K. Agata
Regeneration, 2, 26-36 (2015)
4. Functional joint regeneration is achieved using reintegration mechanism in *Xenopus laevis*.
R. Tsutsumi, S. Yamada and K. Agata
Regeneration, 3, 26-38 (2016)

【Term of Project】 FY2016-2020

【Budget Allocation】 136,800 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.gakushuin.ac.jp/univ/sci/bio/laboratory/detail_agata/theme.html

Title of Project：Molecular basis of pluripotency of vascular stem cells

Hiroo Fukuda
(The University of Tokyo, Graduate School of Science, Professor)

Research Project Number：16H06377　Researcher Number：10165293
Research Area：Biology
Keyword：Plant, stem cell, pluripotency

【Purpose and Background of the Research】

Multicellular organisms have stem cells, which self-proliferate and give rise to various types of differentiated cells. In plants, stem cells in meristems play crucial roles in growth and development. We have studied regulation of stem cell fates in the vascular meristem and found various factors governing stem cell fates such as GSK3 kinases, which are inhibited by bikinin. By using bikinin, we established a new vascular cell differentiation system, Vascular cell Induction culture System Using Arabidopsis Leaves (VISUAL), which induces ectopic differentiation of vascular cells. In this study, therefore, using VISUAL we intend to reveal molecular basis of pluripotency of vascular stem cells.

【Research Methods】

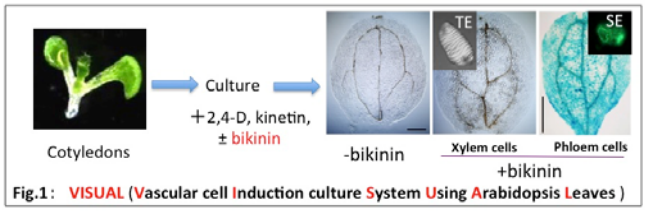


Fig.1：VISUAL (Vascular cell Induction culture System Using Arabidopsis Leaves)

Bikinin induces ectopic differentiation of vascular stem cells (procambial cells) from mesophyll cells and phloem and xylem cells from vascular stem cells in VISUAL (Fig.1). Therefore this system is efficient for studying vascular stem cell fates intensively. Using VISUAL, we aimed at revealing 1) how vascular stem cells are established, 2) how phloem differentiation is initiated, and 3) what determines switching between phloem and xylem differentiation（Fig.2）.

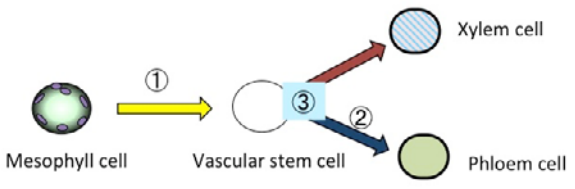


Fig.2：Targets of this study

【Expected Research Achievements and Scientific Significance】

It is expected that this study will reveal the real nature of vascular stem cells and intercellular and intracellular signaling pathways leading to establishment of vascular stem cells and determination of specific vascular cell types. In addition, this study will deepen our understanding of maintenance and development of plant meristems. Comparison with animal stem cells may provide a novel insight into universality and diversity of stem cells in multicellular organisms.

【Publications Relevant to the Project】

- Oda, Y. and Fukuda, H.: Initiation of cell wall pattern by a Rho- and microtubule-driven symmetry breaking. **Science** 337, 1333-1336, 2012
- Kondo, Y., Ito, T., Nakagami, H, Hirakawa, Y., Saito, M., Tamaki, T., Shirasu, K., and Fukuda, H.: Plant GSK3s regulate stem cell differentiation downstream of TDIF-TDR signalling. **Nature Commu**. 5, article number 4505, 2014.
- Kondo, Y., Nurani, A. M., Saito, C., Ichihashi, Y., Saito. M., Yamazaki, K., Mitsuda, N., Ohme-Takagi, M. and Fukuda, H.: Vascular cell Induction culture System Using Arabidopsis Leaves (VISUAL) visualizes the sequential differentiation of sieve element-like cells. **Plant Cell**, in press, 2016.

【Term of Project】 FY2016-2020

【Budget Allocation】 141,800 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.biol.s.u-tokyo.ac.jp/users/seigyo/lab.html

Title of Project : Spatiotemporal regulation of cell division axis as a grand plan of plant developmental evolution

Mitsuyasu Hasebe
(National Institute for Basic Biology, Division of Evolutionary Biology, Professor)

Research Project Number : 16H06378 Researcher Number : 40237996
Research Area : Evolutionary Biology
Keyword : Cell division axis, Evolution and Development, Cell Evolution, Physcomitrella, Closterium

【Purpose and Background of the Research】

Cell division axis has to be properly regulated during development in both metazoans and land plants. Genetic changes in the regulation of cell division axis caused the evolution of development in the multicellular organisms. Since land plants do not have centrosomes and asteroid bodies, both of which are involved in the axis formation of metazoans, land plants should have different regulatory mechanisms. This study aims to investigate the connecting factors between microtubules and GRAS transcription factors that regulate periclinal cell divisions in the moss *Physcomitrella patens*. In addition to identify the factors, the spatiotemporal regulatory mechanisms will be studies to understand the basis of body plan evolution with comparison to those in the flowering plant *Arabidopsis thaliana* and the green algae *Closterium peracerosum–strigosum-littorale*.

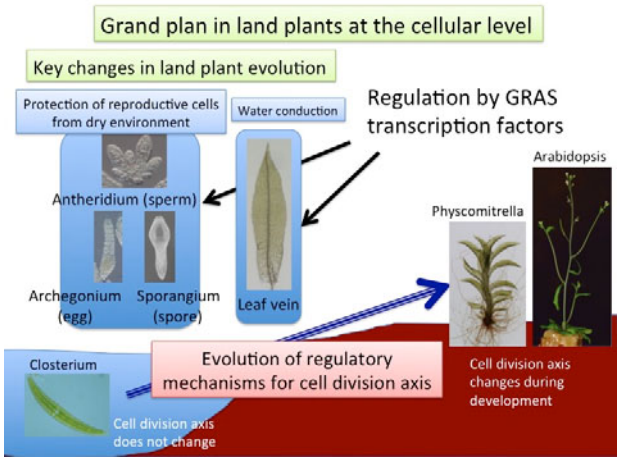


Figure 1 Evolution of the regulatory mechanisms for cell division axis appears to be the basic change for subsequent divergence of land plants

【Research Methods】

This study aims to investigate the molecular mechanisms to spatiotemporally regulate the cell division axis in representative land plants, which enables us to infer the evolutionary significance of the difference of cell division axis regulation.
[Research 1] We investigate target genes of GRAS transcription factors that regulate periclinal cell divisions in Physcomitrella especially focusing on factors connecting the transription factors and microtubules.
[Research 2] Spatiotempral regulation of the GRAS transcription factors during leaf vein development in Physcomitrella.
[Research 3] Based on the results in Researches 1 and 2, functions of orthologous genes to cell division axis regulators in Phsycomitrella will be studied in Arbidopsis and Closterium to investigate their common and different functions and to infer evolutionary significance in body plan evolution.

【Expected Research Achievements and Scientific Significance】

This study will reveal the factors and mechanisms to connect transcription factors and cytoskeleton to spatiotemporally regulate cell division axis in Physcomitrella, Arabidopsis, and Closterium. These results will give new insight on cell biology, developmental biology, as well as evolutionary biology.

【Publications Relevant to the Project】

・Fukushima, K., Fujita, H., Yamaguchi, T., Kawaguchi, M., Tsukaya, H., and Hasebe, M. (2015) Oriented cell division shapes carnivorous pitcher leaves of *Sarracenia purpurea*. Nat. Commun. 6 , 6450
・Kofuji, R. and Hasebe, M. (2014) Eight types of stem cells in the life cycle of the moss *Physcomitrella patens*. Curr. Opin. Plant Biol. 17 , 13-21.

【Term of Project】 FY2016-2020

【Budget Allocation】 150,100 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.nibb.ac.jp/evodevo

Title of Project : Production of Super High-yielding Rice Plants for Environmental Conservation as the Green Evolution II

Amane Makino
(Tohoku University, Graduate School of Agricultural Sciences, Professor)

Research Project Number : 16H06379 Researcher Number : 70181617
Research Area : Plant Nutrition
Keyword : Rice, Photosynthesis, High yields, Biomass, Nitrogen

【Purpose and Background of the Research】

Rice is the most important food crop in the world, accounting for more than 20% of global food production. Breeding of semi-dwarf rice varieties in the 1960s made a great contribution to increasing yield potential, which is called as the Green Revolution. The success in semi-dwarf breeding was caused by photosynthetic enhancement and enlargement of sink capacity depending on large input of N fertilizer. On the other hand, large inputs of N fertilizer in turn have drawn much attention to the environmental impact of N application practices. Therefore, it is important how the yield potential should be increased while limiting the environmental impact of N management practices.

After the success in semi-dwarf breeding, the main targets of rice improvement have moved to the introduction of disease and insect resistance, grain-quality improvements and shortened growth duration. Concerning yield potential, developing hybrid rice and new-plant-type rice with large panicle has been focused. However, there has been no actual increase in the yield potential since the release of the first semi-dwarf cultivars.

The purpose of this study is to construct super high-yielding rice plants with both improvements in source and sink capacities (Figure). To enhance photosynthesis, Rubisco efficiency should be optimized and enhanced. To enlarge sink capacity, the large-grain alleles will be introduced.

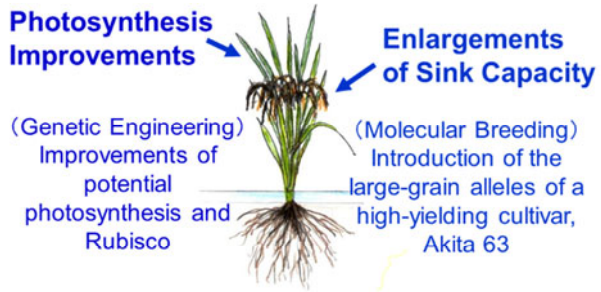


Figure: A super high-yielding rice has higher photosynthetic capacity as well as large sink capacity.

【Research Methods】

We will first produce transgenic rice plants with overproduced key components of electron transport system and Calvin-Benson cycle and Rubisco activase, and then their traits will be introduced into the Rubisco-overproduced rice plants. At the same time, we will also construct near-isogenic lines with the large-grain alleles of a high-yielding cultivar, Akita 63 and crossbreed them with the rice plants with enhanced photosynthetic capacities. Lastly, we will evaluate biomass production and yield of the final rice lines using the isolated fields at the P1P level.

【Expected Research Achievements and Scientific Significance】

The demand for rice food is projected to increase by more than 30% by 2025 because of an exponential increase in the population of Asian and Africa. Therefore, we must construct super high-yielding rice plants as the Green Evolution II while limiting the environmental impact of N management practices.

【Publications Relevant to the Project】

- Sudo E, Suzuki Y and Makino A (2014) Whole-plant growth and N utilization in transgenic rice plants with increased or decreased Rubisco content under different CO_2 partial pressures. *Plant Cell Physiol.* 55: 1905-1911.
- Makino A (2011) Photosynthesis, grain yield and N utilization in rice and wheat. *Plant Physiol.* 155: 125-129.

【Term of Project】 FY2016-2020

【Budget Allocation】 108,300 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.agri.tohoku.ac.jp/syokuei/index-j.html
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Title of Project : Molecular Mechanism and Evolution of Self-Incompatibility in Plants

Seiji Takayama
(The University of Tokyo, Graduate School of Agricultural and Life Sciences, Professor)

Research Project Number : 16H06380　　Researcher Number : 70273836
Research Area : Agricultural Chemistry, Applied Biochemistry
Keyword : Plant Biochemistry, Reproduction, Self-incompatibility

【Purpose and Background of the Research】

Flowering plants have self-incompatibility (SI) systems to avoid inbreeding and to maintain genetic diversity. The self/nonself discrimination in SI is achieved by the interaction between the set of male and female *S*-determinants encoded at the *S*-locus. We have thus far revealed that completely different modes of self/nonself discrimination have been adopted in two plant families, Brassicaceae and Solanaceae (Figure 1).

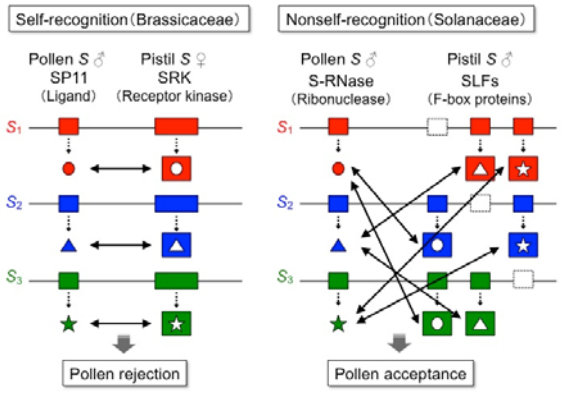

Figure 1. Self- and nonself-recognition in plant self-incompatibility

In the Brassicaceae family, direct and specific interaction between the pollen-localized small ligand protein SP11 and the pistil surface receptor kinase SRK represents the 'self'-recognition, which then induces further downstream events required for self-pollen-rejection.

On the contrary, in the Solanaceae family, a ribonuclease named S-RNase exhibits a specific cytotoxicity against self-pollen, because in the 'nonself'-pollen the S-RNase is detoxified by the pollen F-box proteins (SLFs).

In this research project, we aim to clarify: 1) protein structural basis of the specific self/nonself recognitions; 2) molecular mechanisms of the self-rejection and nonself-acceptance responses caused via the self/nonself discrimination; 3) evolutionary dynamics which lead to the diversification of self/nonself discrimination mechanisms in SI.

【Research Methods】

1) Heterologous protein expression system of SRK, S-RNase and SLFs will be intensively optimized in this project; 2) In the Brassicaceae SI system, revealing the calcium-signaling dynamics in the pistil cells; and in the Solanaceae SI system, fate of S-RNase in the elongating pollen tubes will be investigated in depth; 3) Comparative analysis of the genomic structures will be employed to reveal the evolutionary processes.

【Expected Research Achievements and Scientific Significance】

This research project is expected to advance our understanding of the mechanism of self/non-self discrimination, one of the basic principles of life. The study contributes to revealing diverse physiological processes, as the F-box and receptor-like kinases are the two largest protein families in plants. Studying the evolutionary diversity of SI in plants leads to understand the surviving tactics of plants in fluctuating environments. Scientific advances from this study contribute to establishing new agricultural technologies relevant to F_1 hybrid seed production.

【Publications Relevant to the Project】

・Iwano M, Ito K, Fujii S, *et al.* Calcium signalling mediates self-incompatibility response in the Brassicaceae. **Nature Plants** 1, 15128, 2015.
・Kubo K, Paape T, *et al.* Gene duplication and genetic exchange drive the evolution of S-RNase-based self-incompatibility in *Petunia*. **Nature Plants** 1, 14005, 2015.

【Term of Project】 FY2016-2020

【Budget Allocation】 140,800 Thousand Yen

【Homepage Address and Other Contact Information】

http://bsw3.naist.jp/takayama/
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Title of Project : Comprehensive, Spatiotemporal Study and Applied Research of Carboxydotrophs

Yoshihiko Sako
(Kyoto University, Graduate School of Agriculture, Professor)

Research Project Number : 16H06381 Researcher Number : 60153970
Research Area : Marine microbiology
Keyword : Carboxydotroph, CO metabolism, genomics, marine core, methane hydrate

【Purpose and Background of the Research】

Carboxydotrophic microorganisms can grow on poisonous CO because they possess CO dehydrogenases (CODH), which catalyze the interconversion of CO_2 and CO. CODHs are involved in several metabolic activities such as energy conservation and carbon fixation. Hydrogenogenic carboxydotrophs (CO trophs) possess a CODH gene clustered with hydrogenase genes, and can acquire energy via CO oxidation and H_2 generation. Therefore, hydrogenogenic CO trophs can be considered candidate biocatalysts for improving the efficiency of H_2 production from syngas. Furthermore, CO is the most important precursor for C1-chemistry. CODHs can be new sustainable catalysts for generating CO from CO_2. However, knowledge on diverse CO trophs is limited, and this creates a bottleneck for the development of efficient biocatalysts and catalysts.

Previously, we successfully isolated various thermophilic hydrogenogenic CO trophs from oceanic and terrestrial hydrothermal environments. Of these, one particular bacterium of novel genera, isolated from a core sample of a submerged caldera, harbors six CODH genes. This bacterium appears to be an "ancient-type" CO troph that had been dormant in the core as spores, and is a powerful CO utilizer. Therefore, the aim of this study is to comprehensively understand the CO trophs, particularly "ancient-types", towards construction of a next-generation platform for CO_2 reduction and carbon cycle.

【Research Methods】

(1)We will isolate various CO trophs from water and core samples of oceanic and terrestrial hydrothermal environments and investigate their genetic diversity by metagenomic analysis.
(2)We will unveil CO metabolism of the unique carboxydotrophic isolates by genomic, metabolomic, and transcriptomic analysis.
(3)We plan to characterize recombinants carrying efficient CODH, and construct a large expression system for the CODH.

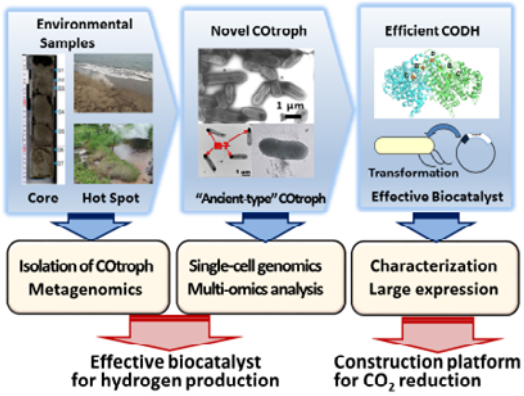


Figure 1 Overall strategy of this study

【Expected Research Achievements and Scientific Significance】

Understanding CO trophs and their CO metabolism will help establish effective heat-resistant biocatalysts for hydrogen production from synthesis gas. Further, we will construct a next-generation platform for CO_2 reduction through development of a sustainable catalyst for conversion of CO_2 to CO.

【Publications Relevant to the Project】

・Yoneda *et al.* (2015) Detection of anaerobic carbon monoxide-oxidizing thermophiles in hydrothermal environments. *FEMS Microbiol. Ecol.* 91: 1-9.
・Yoneda *et al.* (2013) A novel thermophilic, hydrogenogenic, and carboxydotrophic bacterium *Calderohabitans maritimus* gen. nov., sp. nov. from a marine sediment core of an undersea caldera. *Int. J. Syst. Evol. Microbiol.* 63: 3602-3608.

【Term of Project】

FY2016-2020

【Budget Allocation】

133,100 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.microbiology.marine.kais.kyoto-u.ac.jp/

Title of Project : Environmental Interface Engineering Based on Dynamic Analysis of Colloidal Focculation

Yasuhisa Adachi
(University of Tsukuba, Faculty of Life and Environmental Science, Professor)

Research Project Number : 16H06382 Researcher Number : 70192466
Research Area : Agricultural engineering
Keyword : Inhomogeneous colloid, flocculation, sedimentation, electrokinetics, bioresources

【Purpose and Background of the Research】

Any kind of nutrients, minerals and hazardous substances present in nature and water environment, are apt to be adsorbed and to be concentrated on the surface of environmental colloidal particles, such as clay or fine organic matters. This tendency will be enhanced for chemical substances with high hydrophobicity such as dioxin or heavy metals. In addition, these particles will have a tendency to aggregate into large flocs. Under such condition, flocs are more important unit of transportation rather than individual colloidal particles.

The objective of this research is to carry out the systematic analysis of flocculation dynamics focusing that colloidal system in nature and engineered system is composed of nano-particles and dissolved organic matters under the condition of turbulent flow. On the basis of obtained results, the engineering science of colloid and interface in soil and water and biological system will be created and developed in various directions.

【Research Methods】

The three subjects, ①dynamics of flocculation of colloidal particle involved in the adsorption of organic molecules in a flow field ②electrokinetics of porous colloidal complex③sedimentation and rheology of flocculated material, will be placed as core domain, in which advanced analysis of theoretical and experimental aspects are systematically implemented. Obtained results and developed methods, will be utilized in the analysis on ④the solid-liquid separation of concentrated suspension, ⑤flocculation and electrokinetics of the colony of micro-biology, ⑥ formation process of water-quality structure in eco-system. Systematic engineering development will be automatically done throughout these utilization activities（Figure1）. More generally, analogical consideration in the description of colloidal phenomena in the wide domain of bio-resources science will be carried out.

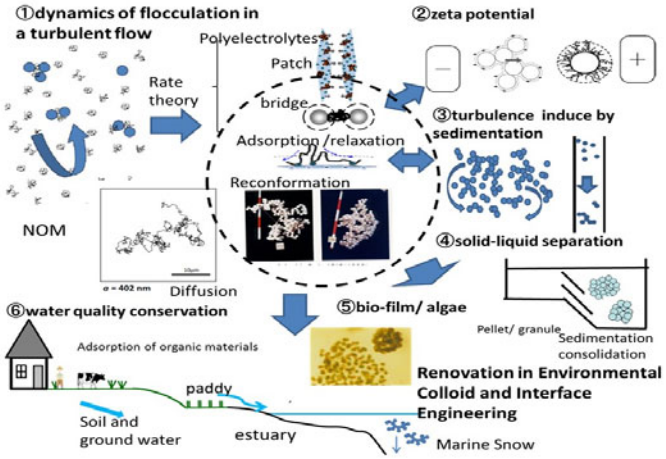


Figure.1 Scheme of Study

【Expected Research Achievements and Scientific Significance】

Understanding of flocculation dynamics is a key factor to clarify the relation between microscopic interaction of colloidal particles and macroscopic transport phenomena. Analysis of the rate theory of flocculation between colloid particles with inhomogeneous surface properties with porous structure is new and will largely contribute to the understanding of more practical system.

【Publications Relevant to the Project】

Tsuchi no koroid gensho - physical chemistry of soil and water environment - ,Yasuhisa Adach, Shingo Iwata, Gakkai Shuppan Center（2003）
Dynamics of polyelectrolyte adsorption and colloidal flocculation upon mixing studied using mono-dispersed polystyrene latex particles, L.Feng, M.A. Cohen Stuart, Y. Adachi, Adv. in Colloid and Interface Sci.226, 101－104（2015）

【Term of Project】FY2016-2020

【Budget Allocation】102,000 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.agbi.tsukuba.ac.jp/~colloid/

Title of Project：Redefinition of intractable inflammatory diseases based on mast cell activation syndrome

Hiroshi Matsuda
(Tokyo University of Agriculture and Technology, Institute of Agriculture, Professor)

Research Project Number：16H06383　Researcher Number：80145820
Research Area：Agriculture
Keyword：Disease prevention and control

【Purpose and Background of the Research】

Mast cells are distributed over the connective tissue widely, and it is well known that a great variety of inflammatory mediators are released from the cells after by binding of pathogen ingredient or IgE antibody to their specific receptors eventually resulting in contribution to the innate immunity or induction of the nonspecific inflammations such as allergic responses.

It is also well known that mast cells located in the microenvironment are associated with the development of intractable inflammatory diseases, but its molecular mechanisms have not been understood by the simple reaction system as stated above. In addition, chemical mediators have material specificity for release kinetics over having many kinds including a species. Released chemical mediators might derive the pathological conditions through peripheral blood in a distant part, and in late years a new concept called "mast cell activation syndrome" was proposed. In this study, I investigate mast cell activation mechanisms involved in the affected sites and identify the functional molecules that induce pathological conditions in a variety of animals suffering from the yet unknown intractable inflammatory diseases. Thus, I do redefinition from a new viewpoint and aim at the development of novel diagnostic methods to identify and evaluate an etiology and pathological conditions of patients and the therapeutic drugs.

【Research Methods】

1) Examine species specificity, tissue specificity, and differentiation specificity of mast cells derived from various animals based on the surface molecules, the quantitative and qualitative data, and the reactive difference with various stimulants. 2) Using various intractable inflammatory disease models, assay chemical mediators derived from mast cells by blood and tissues and evaluate the effectiveness as diagnosis parameters. 3) Clarify the action of the specific chemical mediators from the viewpoint of itch and pain by knock-in or knock-out methods. Furthermore, I identify the target molecules and establish effective control methods of the mast cell activation syndrome.

【Expected Research Achievements and Scientific Significance】

This research project is based on much knowledge provided so far. I focus on an etiology and a process of the aggravation systematically, and the viewpoint that added not only the biochemical point of view but also physicochemical point of view in a species, a local site, the difference at the differentiation stage of mast cells. It clarifies the significant involvement of the mast cell activation syndrome in the development of intractable inflammatory diseases, and it may greatly change the treatment policy by identification of novel effective diagnosis parameters. Social significance is extremely big as well as scientific significance by a companion diagnosis.

【Publications Relevant to the Project】

・Hamilton, M.J. *et al.* Mast cell activation syndrome: A newly recognized disorder with systemic clinical manifestions. J. Allergy Clin. Immunol. 128:147-152 (2011).
・Tanaka, A. *et al.* Mast cells function as an alternative modulator of adipogenesis through 15-deoxy-delta-12, 14-prostaglandin J2. Am. J. Physiol.-Cell Physiol. 301:C1360-C1367 (2011).

【Term of Project】

FY2016-2020

【Budget Allocation】

144,900 Thousand Yen

【Homepage Address and Other Contact Information】

http://web/tuat.ac.jp/~mol_path/
hiro@cc.tuat.ac..jp

Title of Project : Innovative catalysts for the synthesis of large- and medium-sized molecules bearing glycopeptides

Yoshiji Takemoto
(Kyoto University, Graduate School of Pharmaceutical Sciences, Professor)

Research Project Number : 16H06384 Researcher Number : 20227060
Research Area : Pharmaceutical Chemistry, Synthetic Organic Chemistry
Keyword : Synthetic Chemistry, Catalyst, Glycoside, Peptide, Large- and Medium-size Molecule

【Purpose and Background of the Research】

There has been a recent increase in the number of biomedicines being developed based on antibodies and nucleic acids. Most of the approved biological therapeutic agents are manufactured using biological methods because there are very few chemical tools available. However, there are several issues associated with the biological processes currently used, including (1) they cannot be used to prepare structurally pure compounds; (2) biological synthesis processes are expensive; and (3) the development of site-selective chemical modifications is challenging.

To develop efficient drug production processes, as well as therapeutic agents capable of contributing to life science research, there is an urgent need to establish facile, economical, and scalable synthetic methods for the preparation of chemically modified glycopeptides. However, most routine chemical reactions require the addition of excessive amounts of expensive and toxic dehydrating reagents, which leads to large amounts of chemical waste.

The aim of this project is to establish innovative synthetic methods using new catalysts for large- and medium-sized molecules via the site-selective modification of existing compounds composed of amino acids and monosaccharides.

【Research Methods】

The sustainable synthesis of glycopeptides requires the catalytic formation of peptide and glycosyl bonds without using dehydrating agents. Towards this goal, we have designed a series of new catalysts based on the mechanisms associated with the non-ribosomal peptide synthetase- and glycosidase-catalyzed preparation of peptide and oligosaccharides, respectively (Figure 1).

Catalyst **1a** consists of an arylboronic acid, which could activate the carboxylic acid moiety of an amino acid substrate, and a thiol group, which could act as a nucleophile for the subsequent formation of a thioester. We intend to investigate the catalytic efficiency of **1a** in (1) the aza-Michael addition for the synthesis of *N*-alkoxy-α-amino acids and (2) the formation of peptides without any dehydrating agents. It is envisaged that catalyst **1b** could be used to selectively activate the diol units found in monosaccharides using an arylboronic acid. By tuning the second functional group, we will be able to optimize this catalyst for (3) activation-free glycosylation processes and (4) the divergent synthesis of oligosaccharides.

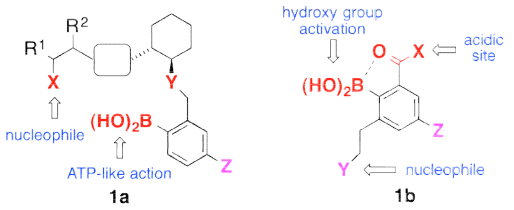


Figure 1 Enzyme-mimetic artificial catalysts

【Expected Research Achievements and Scientific Significance】

This research could result in the development of an environmentally benign manufacturing technology for the preparation of glycopeptides.

The development of new methods for the site-selective modification of glycopeptides will have a considerable impact on the life sciences.

【Publications Relevant to the Project】

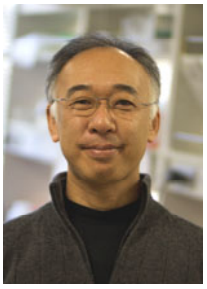
- Hayama, N.; Azuma, T.; Kobayashi, Y.; Takemoto, Y. Chiral integrated catalysts composed of bifunctional thiourea and arylboronic acid: Asymmetric aza-Michael addition of α,β-unsaturated carboxylic acids, *Chem. Pharm. Bull.*, **2016**, *64*, 704-717.
- Azuma, T.; Murata, A.; Kobayashi, Y.; Inokuma, T.; Takemoto, Y., A dual arylboronic acid–aminothiourea catalytic system for the asymmetric intramolecular hetero-Michael reaction of α,β-unsaturated carboxylic acids, *Org. Lett.*, **2014**, *16*, 4256-4259.

【Term of Project】 FY2016-2020

【Budget Allocation】 123,300 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.pharm.kyoto-u.ac.jp/orgchem/
takemoto@pharm.kyoto-u.ac.jp



Title of Project : Mechanisms and physiological functions of intercellular communication by cell death

Masayuki Miura
(The University of Tokyo, Graduate School of Pharmaceutical Sciences, Professor)

Research Project Number : 16H06385 Researcher Number : 50202338
Research Area : Developmental Genetics
Keyword : Inflammation, Cellular Proliferation and cell death, Intercellular communication

【Purpose and Background of the Research】

Function of cell death was thought to be required to get rid of unwanted cells. However, recent genetic study revealed that dying cells release signal molecules and act as a signal center. Our research goal is to reveal the molecular mechanisms and physiological functions of cell-death coupled secretion. Our study will provide the new type of intercellular communication and the novel strategy for diagnosis and treatment of diseases.

【Research Methods】

We have generated FRET sensor for activated caspase-1, named SCAT1. By using SCAT1, the spatial and temporal activation of caspase-1 occurred as the manner of all-or-none (digital) at the single-cell level. Caspase-1 activated macrophage die immediately and real-time concurrent detection of caspase-1 activation and IL-1β release demonstrated that dead macrophages release a local burst of IL-1β in a digital manner. Thus, dying macrophages as the main source of IL-1β within cell populations. To study the molecular mechanisms of caspase-1 mediated active secretion, identification of caspase-1 substrates by Gel-enhanced LC-MS/MS and chemical library screening for IL-1 β secretion will be conducted. We also study the apoptosis- and necrosis-coupled secretion by using *Drosophila* genetics and bioimaging. Three different types of cell death will be focused in our study.

1. Caspase-1 mediated pyroptosis
2. Caspase-3-mediated apoptosis
3. Caspase-independent necrosis

【Expected Research Achievements and Scientific Significance】

Capase-1 activity is required for pyroptosis of macrophage but also for IL-1 β secretion. Identification of the mechanisms of active secretion of signal molecules from three different types of dying cells will provide the novel concept of intercellular communication and contribute for understanding of active participation of cell death for development, tissue homeostasis and diseases.

【Publications Relevant to the Project】

・Kashio, S., Obata, F., Zhang, L., Katsuyama, T., Chihara, T., and Miura, M.: Tissue non-autonomous effects of fat body methionine metabolism on imaginal disc repair in *Drosophila*. Proc. Natl. Acad. Sci. USA., 113, 1835-1840, 2016.
・Yamaguchi, Y., and Miura, M.: Programmed cell death in neurodevelopment.
Dev. Cell 32, 478-490, 2015
・Liu, T., Yamaguchi, Y., Shirasaki, Y., Shikada, K., Yamagishi, M., Hoshino, K., Kaisho, T., Takemoto, K., Suzuki, T., Kuranaga, E., Ohara, O., and Miura, M.: Single-cell imaging of caspase-1 dynamics reveals an all-or-none inflammasome signaling response.
Cell Rep. 8, 974-982, 2014,

【Term of Project】 FY2016-2020

【Budget Allocation】 140,900 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.f.u-tokyo.ac.jp/~genetics/index.html

Title of Project : Signal transduction by transient molecular complexes and its regulation by actin membrane skeleton: single-molecule tracking study

Akihiro Kusumi
(Kyoto University, Institute for Integrated Cell-Material Sciences, Professor)

Research Project Number : 16H06386 Researcher Number : 50169992
Research Area : Single-molecule cellular biophysics, Single-molecule medicinal chemistry
Keyword : Single-molecule tracking, living cells, plasma membrane, meso-scale domains

【Purpose and Background of the Research】

Recently, we have made two totally unanticipated discoveries about the signaling mechanisms common to all of the three experimental paradigms we study (CD59, a prototypical raft-associated receptor; Adrenergic receptor, a prototypical G-protein-coupled receptor; and Fc ε receptor, an immune receptor in mast cells, which is responsible for allergic reactions), which greatly surprised us. Based on these discoveries, we obtained the following two working hypotheses, which we aim to clarify in this project.

(1) Signaling complexes, when directly observed by single-molecule techniques in living cells, are extremely dynamic. Both signaling and scaffolding molecules are constantly exchanging with the dispersed molecules, and the complexes themselves are dynamically forming and dispersing continually, in the time scale often less than 0.1 s. Namely, most of the signaling complexes are extremely transient structures, and are much more dynamic than expected before.

(2) The part of the cortical actin cytoskeleton associated with the PM cytoplasmic surface, called membrane skeleton (MSK), works as the platform for signal transduction in many signaling pathways.

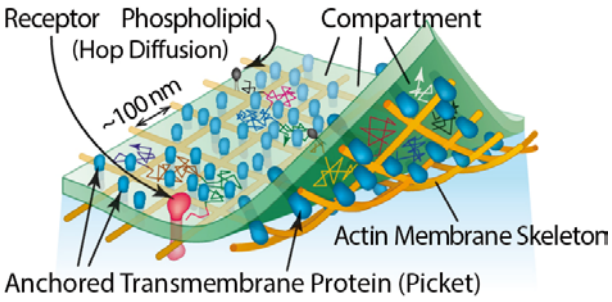


Figure 1. Actin-membrane-skeleton-induced PM compartments.

【Research Methods】

We will systematically examine our working hypothesis, i.e., the gradual minute-order binding/recruitment/activation events observed at the bulk level are induced as the sum of many pulse-like single-molecule events, using the three study paradigms (first in the CD59 signaling). We then reveal the pulse like single-molecule interactions and bulk signals lasting 100~1000 s.

【Expected Research Achievements and Scientific Significance】

We hope to induce a paradigm shift with regard to the mechanisms by which signal transduction in/on the PM is conducted.

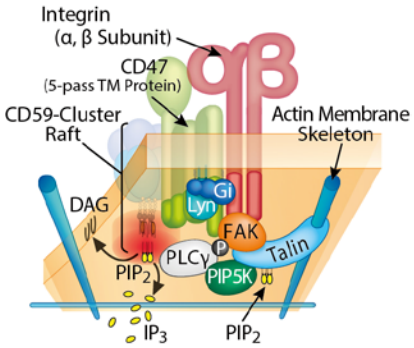


Figure 2. Transient recruitment of cellular signaling molecules to CD59-cluster rafts.

【Publications Relevant to the Project】

A. Kusumi et al. Tracking single molecules at work in living cells (review). Nat. Chem. Biol. 17, 524-532 (2014).

A. Kusumi et al. Organizing principles of the plasma membrane for signal transduction: Membrane mechanisms by the three-tiered hierarchical meso-scale domain architecture. Ann. Rev. Cell Dev. Biol. 28, 215-250 (2012).

【Term of Project】 FY2016-2020

【Budget Allocation】 145,500 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.nanobio.frontier.kyoto-u.ac.jp/index.html

Title of Project：Regulation of innate immune responses by inhibitory immunoreceptors

Akira Shibuya
(University of Tsukuba, Life Science Center for Tsukuba Advanced Research Alliance, Professor)

Research Project Number：16H06387　Researcher Number：80216027
Research Area：Immunology
Keyword：Inhibitory immunoreceptor, innate immunity, disease control

【Purpose and Background of the Research】

The immune system requires regulatory mechanisms that suppresses excessive immune responses. Inhibitory immunoreceptors contains ITIM (immunoreceptor tyrosine-based inhibitory motif) in the cytoplasmic portion and inhibit activating signals by recruiting phosphatases. ITIM-containing inhibitory immunoreceptors PD-1, FcαRIIb and Ly49a expressed on T, B, NK cells, respectively, were identified and found to be involved in regulation of adaptive immune responses. However, regulatory mechanisms of innate immunity by inhibitory immunoreceptors remains incompletely understood.

We identified ITIM-containing inhibitory immunoreceptors MAIR-I, Allergin-1, and Clec10a expressed on dendritic cells, macrophages, and mast cells (Figure 1). In this study, we clarify the regulatory mechanisms of innate immune responses by these inhibitory immunoreceptors. This study on immunological function of these inhibitory immunoreceptors will facilitate understanding of negative regulation of innate immune responses and clarify the pathophysiological significance of the these immunoreceptors. Based on the results in this study, we aim to develop the molecular target therapy against MAIR-I, Allergin-1, and Clec10a for treatment of infection, allergy and inflammation.

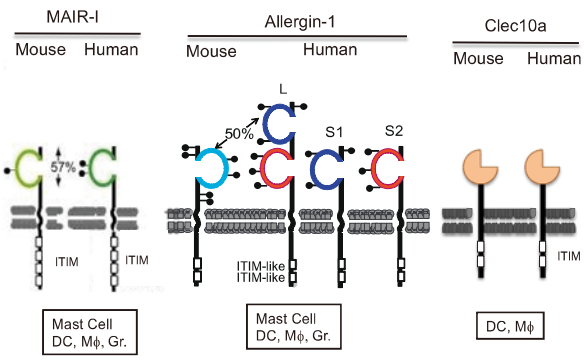


Figure 1. The structure of inhibitory immunetors

【Research Methods】

We will identify the ligands for MAIR-I, Allergin-1, and Clec10a by using Fc-fused soluble chimeric proteins and analyze the spatio-temporal interaction between these inhibitory immunoreceptors and the ligands. Further, we will establish conditional knock-out mice and analyze the function of these inhibitory immunoreceptors expressed on immune cells involved in innate immune responses. Moreover, we clarify the role of the inhibitory immunoreceptors in the pathogenesis of infection, allergy and inflammation by using disease mouse model and develop molecular target therapy by generating antagonistic or agonistic antibodies or proteins for these inhibitory immunoreceptors.

【Expected Research Achievements and Scientific Significance】

The immune system discriminate self and non-self and attack non-self, while protect self (self-tolerance). This study will clarify the positive and negative regulatory mechanisms of innate immune responses, which has remained incompletely understood, and lead to the development of new therapy to intractable diseases.

【Publications Relevant to the Project】

1. Nakahashi-Oda C, et al. Apoptotic epithelial cells control regulatory T cell expansion. ***Nat Immunol***, 17:441-50, 2016
2. Hitomi K, et al. An immunoglobulin-like receptor, Allergin-1, inhibits immunoglobulin E–mediated immediate hypersensitivity reactions. ***Nat Immunol***, 11:601-607, 2010

【Term of Project】 FY2016-2020

【Budget Allocation】 142,600 Thousand Yen

【Homepage Address and Other Contact Information】

http://immuno-tsukuba.com/index.html

Title of Project : The study on the molecular and cellular bases underlying the crosstalks between innate immunity and cell metabolism in lysosomes

Kensuke Miyake
(The University of Tokyo, Institute of Medical Science, Professor)

Research Project Number : 16H06388　Researcher Number : 60229812
Research Area : Immunology
Keyword : Innate Immunity

【Purpose and Background of the Research】

Toll-like receptors (TLRs) sense microbial products and induces defense responses. Nucleic acid (NA) is a principal ligand. Self-derived NA activates TLRs and induces a variety of autoimmune diseases. NA-sensing TLRs are localized in lysosomes.

NAs are continuously degraded in lysosomes and TLR responses to self NAs are therefore prevented in unperturbed condition. DNA degradation by DNase II, however, is required for DNA sensing by DNA-sensing TLR9. Furthermore, RNA-sensing TLR7 and TLR8 respond to ribonucleosides such as uridine and guanosine, suggesting requirement of RNA processing from RNA into ribonucleosides in RNA sensing by TLR7 and TLR8.

The mammalian target of rapamycin (mTOR) is a metabolic sensor. Although TLRs and mTOR are both localized in lysosomes, little is known about their relationship.

The present study focuses on the relationship between NA sensing by TLRs and NA metabolism in lysosomes and the relationship between TLRs and mTOR.

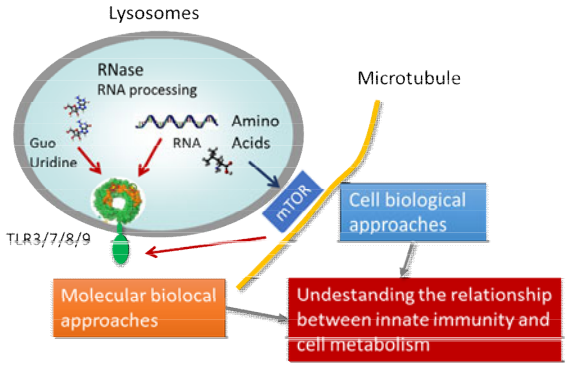


Figure 1: Understanding the crosalks between innate immunity and cell metabolism in lysosomes

【Research Methods】

To study the relationship between NA metabolism and NA sensing, molecules involved in RNA degradation and processing will be studied with particular focus on RNases. Diseases caused by loss of function of those molecules are also to be studied.

To understand the crosstalks between NA-sensing TLRs and mTOR, molecular mechanism underlying type I IFN production by TLRs is to be studied with particular focus on TLR trafficking.

【Expected Research Achievements and Scientific Significance】

The present study aims to reveal the relationship between NA metabolism and NA sensing by TLRs in lysosomes. The finding from the present study would contribute to our understanding on the pathophysiological mechanism underlying a variety of autoimmune diseases.

After sensing NAs, TLRs activate innate immune responses. This decision needs to be made under the permission from cell metabolism. The present study would reveal the mechanism by which metabolic cues are integrated into innate immune responses. In this mechanism, we believe that TLR trafficking plays an important role. Our results would have an impact on not only immunology but also cell biology.

【Publications Relevant to the Project】

・Chan MP, Onji M, Fukui R, Kawane K, Shibata T, Saitoh SI, Ohto U, Shimizu T, Barber GN, Miyake K. DNase II-dependent DNA digestion is required for DNA sensing by TLR9. ***Nat Commun***. 2015 6:5853.
・Shibata T, Ohto U, Nomura S, Kibata K, Motoi Y, Zhang Y, Murakami Y, Fukui R, Ishimoto T, Sano S, Ito T, Shimizu T, Miyake K. Guanosine and its modified derivatives are endogenous ligands for TLR7. ***Int Immunol***. 2016 28:211-222.

【Term of Project】 FY2016-2020

【Budget Allocation】 140,900 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.ims.u-tokyo.ac.jp/kanseniden/index.html

Title of Project : Elucidation of the mechanism required for AIM activation, and its therapeutic application to NASH-induced hepatocellular carcinoma

Toru Miyazaki
(The University of Tokyo, Faculty of Medicine, Professor)

Research Project Number : 16H06389 Researcher Number : 30396270
Research Area : Gastroenterology
Keyword : NASH-HCC, AIM, Fatty liver, Obesity

【Purpose and Background of the Research】

Non-alcoholic steatohepatitis (NASH) is a manifestation of metabolic syndrome in the liver, and is a potent risk factor for the development of hepatocellular carcinoma (HCC). The number of patients of NASH as well as NASH-HCC is increasing recently. Previously, we showed that the circulating protein AIM exhibits strong anti-HCC effect brought about by complement activation. Alongside the therapeutic use of recombinant AIM protein, we here aim to develop the system that activates blood AIM through its dissociation from IgM pentamer, and to apply it for the therapy of NASH-HCC. We will also establish a system to evaluate IgM-free AIM levels in serum, and use it for early diagnosis and prognosis prediction of NASH-HCC.

【Research Methods】

(1) Exploration of mechanism for AIM activation: In healthy states, AIM associates with IgM-pentamer, which stabilizes but inactivates AIM. However, AIM converts to active form via dissociating from IgM under various disease states including acute kidney injury (AKI). First, we will investigate the "physiological activator(s)" which are induced during AKI and dissociate AIM from IgM. In addition, we will search "chemical (artificial) activators" such as specific peptides or small compounds, which interact with the binding site of AIM responsible to binding with IgM, and lead to AIM-IgM dissociation (Figure 1).

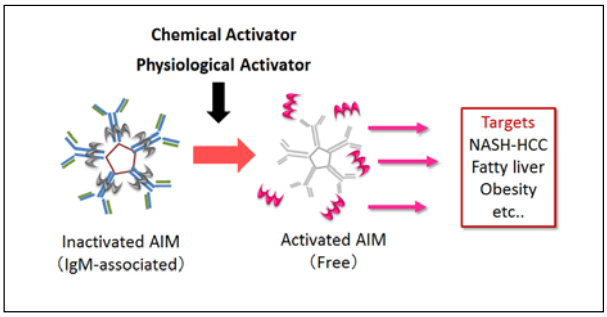


Figure 1 Activation of endogenous AIM

(2) Application of AIM activators for NASH-HCC therapy: Physiological and Chemical activators obtained in (1), will be tested for their anti-HCC effect using animal disease models towards the future drug development for human disease.

(3) Global analysis of serum IgM-free AIM (= active AIM) levels in patients: Blood samples from patients with liver diseases including simple steatosis, NASH, and NASH-HCC, will be analyzed for total and active AIM levels to investigate their relations with disease progression. Through this study, we would like to achieve early diagnosis of NASH-HCC by AIM levels.

【Expected Research Achievements and Scientific Significance】

We have demonstrated marked therapeutic effects of AIM administration against NASH-HCC. However, the large scale production of functional AIM protein possesses some technical difficulties due to its complicated structure caused by its many cysteine residues, and thus, demands a high cost. This problem will be overcome by activating endogenous AIM, which are present abundantly in blood. Our study will be the basis for the development of novel therapeutic and diagnostic tools for NASH-HCC.

【Publications Relevant to the Project】

・Arai S, Kitada K, et al, Apoptosis inhibitor of macrophage protein enhances intraluminal debris clearance and ameliorates acute kidney injury in mice. Nat Med 22:183-193, 2016
・Maehara N, Arai S, et al, Circulating AIM prevents obesity-associated hepatocellular carcinoma through complement activation. Cell Rep. 9:61-74, 2014

【Term of Project】 FY2016-2020

【Budget Allocation】 133,800 Thousand Yen

【Homepage Address and Other Contact Information】

http://tmlab.m.u-tokyo.ac.jp/english/index.html
E-mail: tm@m.u-tokyo.ac.jp

Title of Project : Elucidation of lifestyle-related diseases development due to environmental factors and epigenetic memory

Juro Sakai
(The University of Tokyo, Research Center for Advanced Science and Technology, Professor)

Research Project Number : 16H06390 Researcher Number : 80323020
Research Area : Metabolic Medicine, Molecular biology
Keyword : metabolic syndrome, epigenome, signal transduction

【Purpose and Background of the Research】

Metabolic syndrome associated with obesity and its related metabolic disorders such as type 2 diabetes and hyperlipidemia is a major challenge of the 21st century biomedical. Epigenomic gene regulation is an adaptive mechanism to the environment changes and is deeply involved in the lifestyle-related diseases. However, how the external cues determine the specific epigenomic changes were not clearly understood.

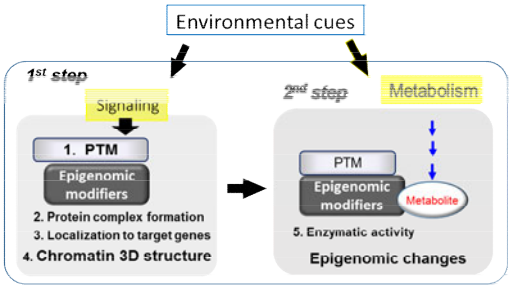
We recently revealed that post-translational modification (PTM) of histone modification enzyme and its consequent protein complex formation (1st step) is the key step to determine specify (Figure). In this study, we elucidate the mechanisms of 1st step further and elucidate mechanisms of consequent histone modification changes (2nd step). This 2nd step rewrites epigenome and ensures sustained and stable gene expressions and may relate to the predisposition to life style diseases under certain environment and nutrition conditions. Through these, we aim to develop innovative and effective treatment of lifestyle-related diseases.

【Research Methods】

Using metabolomics together with epigenomic analyses, we elucidate the mechanisms of "re-writing" of epigenome. We analyze metabolome and how nutrition and metabolites regulates histone modification enzymes and consequently rewrite epigenome. We will reveal further the JMJD1A-Ser265 phosphorylation protein complex to elucidate p-Ser265 mediated thermogenesis and browning of fat cells. We generates S265A knock-in mice and analyze the phenotype, especially the browning of fat cells. We further analyze AMPK mediated phosphorylation of JMJD1A, its phosphorylation sites, and its roles in energy metabolism. We evaluate the role of histone methyltransferase SETDB1 by elucidating the ubiquitination and the protein complex for E3 ubiquitin ligase and deubiquitinating enzymes. Thereby we reveal the role of SETDB1 in previously we identified H3K9/H3K4 bivalent chromatin.

【Expected Research Achievements and Scientific Significance】

To inhibit protein phosphatase complex for phospho-JMJD1A would be effective to maintain P-JMJD1A-PPARγ complex thereby activates thermogenic gene program. In addition, by elucidating 2 sequential model for epigenomic memory (Figure), drug design and discovery for histone modification enzymes would become more specific by aiming to the 1st step that determines the gene targets and protein complex. In addition, by elucidating the 2nd step, we could develop new approach to change predisposition to obesity and metabolic syndrome by reprograming epigenome.



Epigenetic memory

【Publications Relevant to the Project】

・Matsumura Y. et al. (2015) H3K4/H3K9me3 Bivalent Chromatin Domains Targeted by Lineage-specific DNA Methylation Pauses Adipocyte Differentiation. **Molecular Cell**, 60, 584-596,
・Abe Y, et al. (2015) JMJD1A is a signal-sensing scaffold that regulates acute chromatin dynamics via SWI/SNF association for thermogenesis. **Nature Commun**, 6, 7052

【Term of Project】 FY2016-2020

【Budget Allocation】 140,700 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.mm.rcast.u-tokyo.ac.jp

Title of Project : Regulation of self-renewal vs. quiescence status in human myeloid leukemia stem cells

Koichi Akashi
(Kyushu University, Graduate School of Medicine, Professor)

Research Project Number : 16H06391 Researcher Number : 80380385
Research Area : Biological Sciences
Keyword : Hematology

【Purpose and Background of the Research】

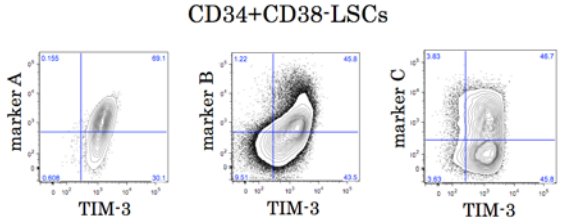
Acute myeloid leukemia (AML) originates from self-renewing leukemic stem cells (LSCs). Purified human LSCs can repopulate human AML in immunodeficient mice, and therefore, LSCs should be an ultimate therapeutic target for treatment of AML. LSCs can self-renew to expand, but can also stop cell cycle to escape chemo-radiotherapy. However, the molecular mechanisms regarding how LSCs dramatically change their cellular status are still unknown. In this project, we will clarify the molecular mechanisms of the plasticity of LSCs.

【Research Methods】

The T-cell immunoglobulin mucin-3 (TIM-3) is expressed on the surface of LSCs in the majority of AML patients (Kikushige et al., Cell Stem Cell 2010). TIM-3 and its ligand, galectin-9 formed an autocrine loop critical for LSC maintenance. TIM-3+ LSCs of human myeloid malignancies secrete galectin-9 into sera, and it binds to TIM-3 to induce self-renewal signaling (Kikushige et al., Cell Stem Cell 2015). The TIM-3/galectin-9 autocrine loop is universally used in LSCs of a variety of myeloid malignancies including AML, myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPN) to achieve the clonal dominancy during their disease progression process.

We have identified novel LSCs-specific surface molecules based on the transcriptome analysis of human $CD34^+CD38^-$ LSCs (Figure 1), and some of these molecules are specifically expressed in quiescent LSCs. By using combination of antibodies against TIM-3 and new surface antigens, we will try to isolate new subpopulations of LSCs with different functions, and will analyze biological characteristics of these subpopulations. We will test the LSC activity of these subpopulations by utilizing a new immuno-deficient xenograft model, and by using high throughput omics analyses including exome sequencing, RNA sequencing and metabolome analysis. We will try to reveal the molecular mechanisms of heterogeneity in human myeloid LSCs, and the role of microenvironment in maintenance of their heterogeneity.

figure 1



These markers enables us to prospectively isolate new sub-fraction of TIM-3+ LSCs

【Expected Research Achievements and Scientific Significance】

We will establish the method for purification of LSC subpopulation with distinct stem cell activities to understand regulation of self-renewal vs. quiescence status in human myeloid malignant stem cells. The result will help understand how LSCs become resistant to chemo-radiotherapy, and to develop new treatment strategies targeting LSCs.

【Publications Relevant to the Project】

1. Y. Kikushige *et al.*, TIM-3 is a promising target to selectively kill acute myeloid leukemia stem cells. *Cell Stem Cell* **7**, 708 (Dec 3, 2010).
2. Y. Kikushige *et al.*, A TIM-3/Gal-9 Autocrine Stimulatory Loop Drives Self-Renewal of Human Myeloid Leukemia Stem Cells and Leukemic Progression. *Cell Stem Cell* **17**, 341 (Sep 3, 2015).

【Term of Project】

FY2016-2020

【Budget Allocation】

118,500 Thousand Yen

【Homepage Address and Other Contact Information】

http:// www.1nai.med.kyushu-u.ac.jp

Title of Project : Elucidation of the molecular mechanism of homeotic control of muscle and bone from the viewpoints of inter-organ crosstalk

Shu Takeda
(Tokyo Medical and Dental University, Graduate School of Medical and Dental Sciences, Professor)

Research Project Number : 16H06392 Researcher Number : 30376727
Research Area : Orthopedic Surgery
Keyword : Bone and cartilage metabolism

【Purpose and Background of the Research】

Age-related decrease in muscle mass and muscle function, i.e. sarcopenia, has been to lead decrease in motor ability and eventually shorten the healthspan. However, its pathogenesis is unknown and new medical treatment is strongly awaited. In addition, detailed molecular mechanism of bone formation is largely unknown. Novel regulatory mechanism of metabolism by the functional communication between organs or tissues, i.e., inter-organ network, such as cardio-renal axis or adipo-vascular axis, draws great attention, indicating that a novel regulatory function can be discovered by the development of research project focusing inter-organ network. We previously demonstrated that hormones and neuropeptides, such as leptin and NMU, regulated bone remodeling through the central nervous system and proposed the concept "control of bone by organs other than bone". Moreover, we also demonstrated that sensory neurons are physiologically essential for the homeostasis of bone remodeling.

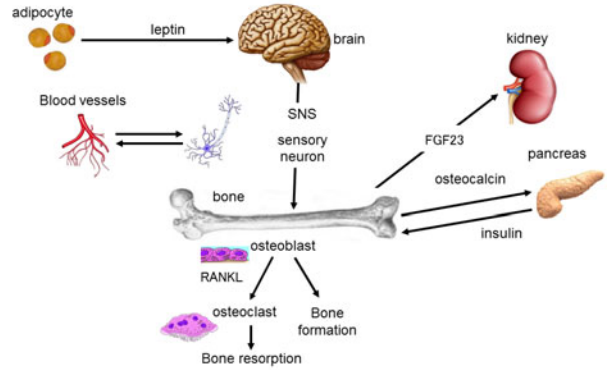
In this research project, we will study the role of neurons and blood vessels for the regulation of muscle and osteochndro progenitor cells and aimed to identify regulatory factors for these progenitors and apply them for novel therapeutics.

【Research Methods】

We will analyze neurons and blood vessels innervating muscles and bones in three dimensions by using newly developed techniques that make the tissues transparent. By using tissue-specific Sema3a receptor-deficient mice, we will also analyze the role of neurons and blood vessels for the regulation of muscle and osteochndro progenitor cells and aimed to identify regulatory factors for these progenitors and apply them for novel therapeutics.

【Expected Research Achievements and Scientific Significance】

This research project will further develop the concept "control of metabolism by inter-organ network" and enable us to address the molecular mechanism of neuron-dependent control of muscle and osteochondro stem cells. In addition, this project is expected to lead to develop novel therapeutics for the treatment of sarcopenia and osteoporosis.



Inter-organ metabolic crosstalk between bone and other organs

【Publications Relevant to the Project】

・Takeda, S., Elefteriou, F., Levasseur, R., Liu, X., Zhao, L., Parker, K.L., Armstrong, D., Ducy, P., and Karsenty, G. Leptin regulates bone formation via the sympathetic nervous system. Cell 111:305-17, 2002

・Fukuda, T., Takeda, S., Xu, R., Ochi, H., Sunamura, S., Sato, T., Shibata, S., Yoshida, Y., Gu, Z., Kimura, A., Ma, C., Xu, C., Bando, W., Fujita, K., Shinomiya, K., Hirai, T., Asou, Y., Enomoto, M., Okano, H., Okawa, A., and Itoh, H. Sema3A regulates bone-mass accrual through sensory innervations. Nature 497:490-3, 2013

【Term of Project】 FY2016-2020

【Budget Allocation】 150,300 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.med.tmd.ac.jp/medicine/list/basic/functional/cell_physiology.html

Title of Project : Development of innovative medical technology based on integrated understanding of both protection and destruction of articular cartilage homeostasis

Riko Nishimura
(Osaka University, Graduate School of Dentistry, Professor)

Research Project Number : 16H06393 Researcher Number : 60294112
Research Area : Oral Basic Science, Biochemistry, Molecular Biology
Keyword : Articular chondrocyte, Transcription factor, Osteoarthritis, Regeneration

【Purpose and Background of the Research】

When articular chondrocytes and their matrices are impaired by aging, inflammation, and excessive mechanical stress, cartilage diseases such as osteoarthritis occur. Because of super-aged society in Japan, patients of osteoarthritis are increasing to approximately twenty millions.

In articular cartilage lesions destroyed by osteoarthritis, histological phenomena and gene expression patterns resembled to hypertrophy of growth plate chondrocytes and the subsequent events including destruction of cartilage matrices are observed. Therefore, investigation of mechanisms of osteoarthritis have been performed based on molecular mechanisms of hypertrophy of chondrocytes and destruction of cartilage matrices.

Recently, it is getting clearer that articular chondrocytes have different properties from growth plate chondrocytes. However, it is still elusive what cellular and molecular properties articular cartilage chondrocytes have.

In this project, we have planned to characterize articular cartilage chondrocytes at cellular and molecular levels, understand molecular mechanisms how articular cartilage chondrocytes retain their homeostasis, investigate the signal resulted in osteoarthritis and reveal molecular pathogenesis of osteoarthritis. Moreover, we attempt to contribute to development of novel effective therapy and early diagnostic method for osteoarthritis.

【Research Methods】

1. It is very important for understanding of property of articular chondrocytes to identify specific transcription factors involved in regulation of articular chondrocytes homeostasis. We are planning to isolate them by performing enChIP cloning method and microarray analyses of articular chondrocytes.

2. We are attempting to identify transcription factors involved in osteoarthritis, based on human osteoarthritis data base. Subsequently, we will investigate functional roles of the transcription factors in vitro as well as in vivo.

3. We will analyze gene expression profiles of articular chondrocytes by performing microarray analyses, and then attempt to isolate molecules involved in articular cartilage homeostasis. After identification, we will examine their functional roles in articular cartilage by performing knockdown and/or Cas9 knockout systems against the genes.

【Expected Research Achievements and Scientific Significance】

We are expecting to open up articular cartilage biology, and develop new technologies that led to a paradigm shift in treatment of osteoarthritis.

【Publications Relevant to the Project】

・Hata K, Takashima R, Amano K, Ono K, Nakanishi M, Yoshida M, Wakabayashi M, Matsuda A, Maeda Y, Suzuki Y, Sugano S, Whitson R, Nishimura R, Yoneda Y (2013) Arid5b facilitates chondrogenesis by recruiting the histone demethylase Phf2 to Sox9-regulated genes. ***Nature Communications.*** 4: 2850 DOI: 10.1038/ncomms3850

・Yoshida M, Hata K, Takashima R, Ono K, Nakamura E, Takahata Y, Murakami T, Iseki S, Takano-Yamamoto T, Nishimura R, Yoneda T. (2015) The transcription factor Foxc1 is necessary for Ihh-Gli2-regulated endochondral ossification. ***Nature Communications*** 6: DOI: 10.1038/ncomms7653.

【Term of Project】

FY2016-2020

【Budget Allocation】

139,900 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.dent.osaka-u.ac.jp/admission/admission_000294.html