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ANNUAL REPORT 2018

Medical Research Institute / Tokyo Medical and Dental University

Annual Report 2018

Tokyo Medical and Dental University

2018

Annual Report

Medical Research Institute

Tokyo Medical and Dental University

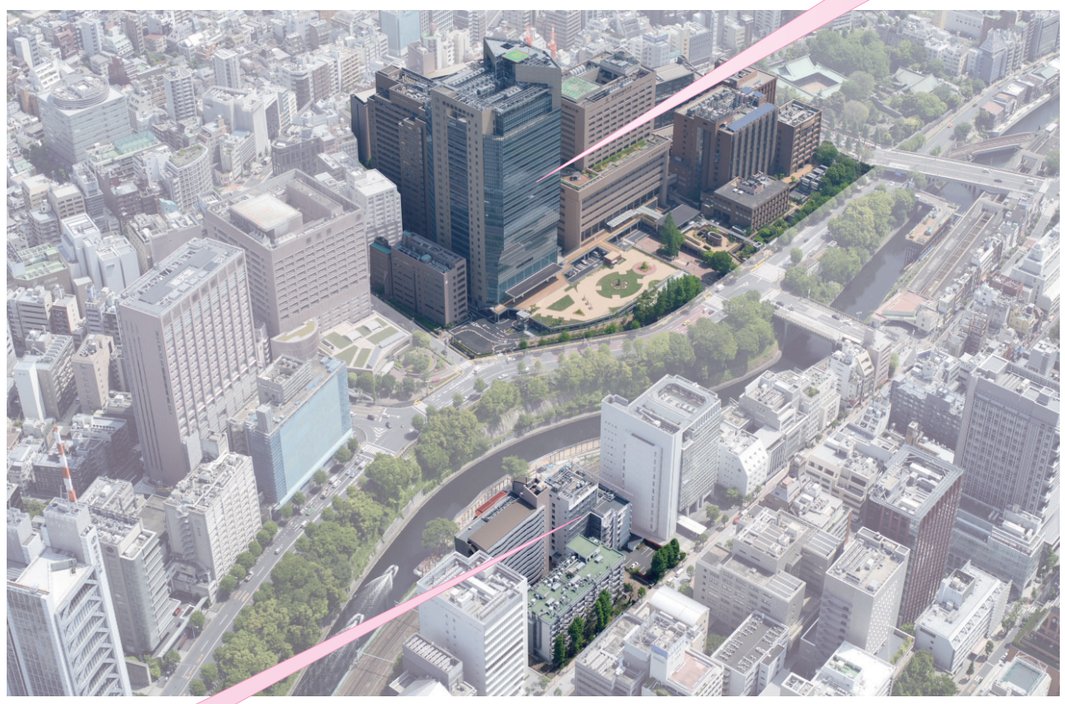
Contents

Medical Research Institute

Yushima Area

1-5-45 Yushima Bunkyo-ku Tokyo 113-8510 Japan

Medical Research Institute
Department of Molecular Neuroscience, Department of Pathological Cell Biology, Department of Developmental and Regenerative Biology, Department of Immunology, Department of Molecular Cytogenetics, Department of Molecular Genetics, Department of Medical Science Mathematics, Department of Molecular Cell Biology, Department of Bio-informational Pharmacology, Department of Molecular Pathogenesis, Department of Epigenetics, Department of Stem Cell Regulation, Department of Neuropathology, Department of Structual Biology, Department of Biodefense Research, Department of Stem Cell Biology, Department of Genomic Pathology, Department of Molecular Epidemiology, Frontier Research Unit Laboratory of Gene Expression, Frontier Research Unit Redox Response Cell Biology, Frontier Research Unit Laboratory of Oxygen Biology, Tenure Track Research Unit Department of Cellular and Molecular Medicine, Administrative Office



Surugadai Area

2-3-10 Kanda-surugadai Chiyoda-ku Tokyo 101-0062 Japan

Medical Research Institute
Project Research Unit

Medical Research Institute

Director
- Fumitoshi Ishino
- Faculty Meeting

Research Divisions

Division of Advanced Molecular Medicine

Department of Molecular Medicine and Metabolism	(under consideration)	
Department of Molecular Pharmacology	(under consideration)	
Department of Molecular Cell Biology	Professor	Hiroshi Shibuya
Department of Molecular Neuroscience	Professor	Kohichi Tanaka
Department of Biodefense Research	Professor	Toshiaki Ohteki
Department of Bio-informational Pharmacology	Professor	Tetsushi Furukawa
Department of Stem Cell Regulation	Professor	Tetsuya Taga
Department of Structual Biology	Professor	Nobutoshi Ito
Frontier Research Unit Laboratory of Oxygen Biology		
Frontier Laboratory: Skeletal Molecular Pharmacology		

Division of Pathophysiology

Department of Neuropathology	Professor	Hitoshi Okazawa
Department of Biochemical Pathophysiology	Professor	Takehiko Sasaki
Department of Pathological Cell Biology	Professor	Shigeomi Shimizu
Department of Developmental and Regenerative Biology	Professor	Hiroshi Nishina
Department of Stem Cell Biology	Professor	Emi K. Nishimura
Department of Immunology	Professor	Takeshi Tsubata
Department of Molecular Pathogenesis	Professoror	Akinori Kimura

Division of Medical Genomics

Department of Molecular Cytogenetics	Professor	Johji Inazawa
Department of Molecular Genetics	Professor	Yoshio Miki
Department of Molecular Epidemiology	Professor	Masaaki Muramatsu
Department of Biochemical Genetics	(under consideration)	
Department of Genomic Pathology	Professor	Shumpei Ishikawa
Department of Epigenetics	Professor	Fumitoshi Ishino
Department of Medical Science Mathematics	Professor	Tatsuhiko Tsunoda
Frontier Research Unit Laboratory of Gene Expression		
Project Reserch Unit		

Division of Integrative Research

System Biology for Intractable Diseases	Visiting Professors	Satoru Miyano and Seiya Imoto

Laboratory for Integrated Research Projects on Intractable Diseases

Advanced Technology Laboratory

- Genome Laboratory
- Laboratory of Cytometry and Proteome Research
- Laboratory of Recombinant Animals
- Laboratory of Anatomy and Cell Function
- Bioresource Laboratory
- Laboratory of Structural Biology
- Stem Cell Laboratory

Administrative Office

- General Affairs Section

Highlight

Discovery of human monocyte/macrophage progenitors

Kawamura S, et al., Identification of a human clonogenic progenitor with strict monocyte differentiation potential – a counterpart of mouse cMoPs. ***Immunity*** 46, 835-48 (2017).

In the recent decade, macrophage-related studies have revealed that the majority of tissue-resident macrophages are derived from embryonic erythroid-myeroid progenitors (EMP), and inflammation prompts tissue-infiltration of bone marrow (BM)-derived monocytes and their differentiation into macrophages to gradually replace the tissue-resident macrophages. In inflammatory bowel disease (IBD), BM-derived monocytes are recruited into the intestinal mucosa, where they become colitogenic macrophages producing TNF α and extend the inflammatory pathology. In cancer tissue, they become tumor-associated macrophages (TAM), thereby directly promoting tumor growth, invasion and metastasis. On the other hand, TAM suppresses immune cells including cytotoxic T cells. In addition, monocytes and monocyte-derived macrophages cause bone diseases including osteoporosis, metabolic syndrome and fibrosis.

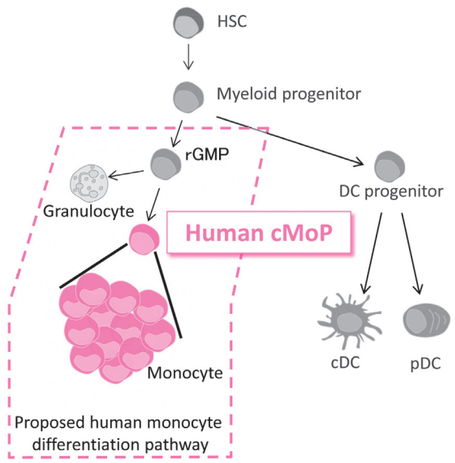


Fig.1 Discovery of human cMoPs

Monocytes arise from hematopoietic stem cells (HSCs) via sequential intermediate progenitors in the BM. In mice, a common monocyte progenitor (cMoPs) that is restricted to the monocyte lineage was identified in 2013. In humans, however, it remained largely unknown about the origin of monocytes and the presence of monocyte-committed progenitors etc.

To identify human cMoPs, we focused on previously reported granulocyte and monocyte progenitors (GMPs) as an upstream progenitor of cMoPs. Using Fc-RI (CD64) and C-type lectin CLEC12A, which had been selected after screening a variety of cell-surface markers expressed on monocytes and macrophages, we succeeded to divide conventional GMPs into four subpopulations. Among them, we found human cMoPs as CLEC12AhiCD64hi cells. The presence of cMoPs within the conventional GMP population implies that conventional GMPs are a mixed population of genuine GMPs and other progenitors, and this finding led us to redefine human GMPs as a subset of CLEC12AhiCD64int cells within the conventionally defined GMP population. This subset of revised GMPs gave rise sequentially to cMoPs, pre-monocytes, and monocytes (Fig.1). Consistently, the hierarchical tree and plot flow of each population based on principle component (PC) 1 analysis fit with the sequential monocyte differentiation process.

Collaboration with pharmaceutical company toward the development of therapeutic agents targeting cMoP and monocyte lineage is currently in progress.

Inter-University Research Network for Trans-Omics Medicine

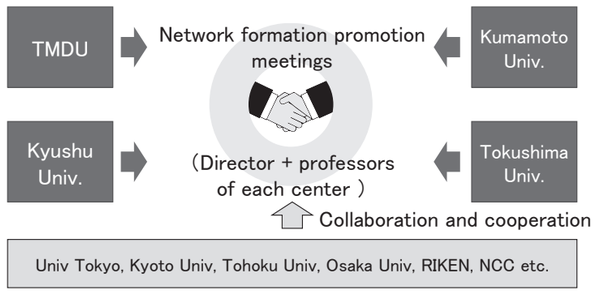
Since April, 2016, Medical Research Institute at Tokyo Medical and Dental University promotes the "Inter-University Research Network for Trans-Omics Medicine Project" aiming to establish a trans-omics research education hub. We are carrying out this project in cooperation with Kyushu University, Kumamoto University, Tokushima University's joint use and collaborative research centers with the support of the Ministry of Education culture, sports, Science and Technology.

Aim of the Project

* In order to realize trans-omics research, promote domestic technology development, human resource development and establish a research platform.
* Although various omics studies have been established from now on, technologies and experts who integrate different kinds of big data are required. Four domestic research centers with outstanding achievements work together to solve this urgent issue ahead of the world.

Participating Joint Usage/Research Centers

* Medical Research Institute, Tokyo Medical and Dental University (TMDU) (Joint Usage/Research Center for Intractable Diseases)
* Medical Institute of Bioregulation, Kyushu University (Research Center for Multi-Scale Research of Host Defense Systems)
* Institute of Advanced Medical Sciences, Tokushima University (Joint Research Core Network Institute for Enzyme Research)
* Institute of Molecular Embryology and Genetics, Kumamoto University (Joint Usage/Research Center for Developmental Medicine)



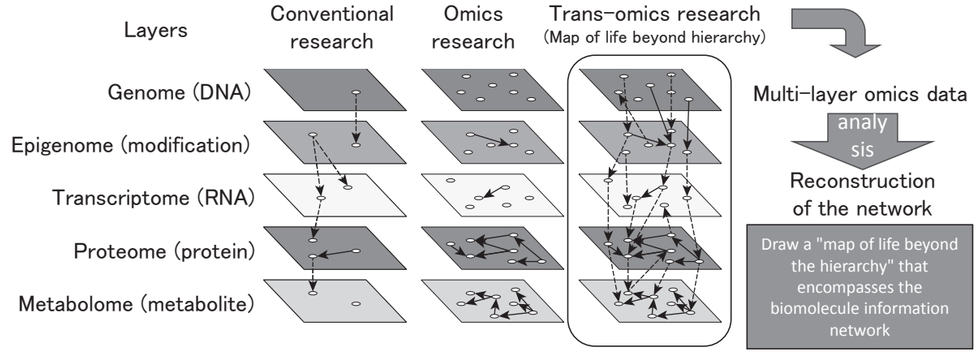
Activities

Joint research symposium

* Co-sponsored symposium "Frontiers in Biomedical Sciences"
  Date: January 26-27, 2017
  Place: Conference room, Institute of Molecular Embryology and Genetics, Kumamoto University
* Co-sponsored symposium "The 291st IMEG Seminar Tokyo Medical and Dental University- IMEG Joint Seminar"
  Date: February 24, 2017
  Place: Conference room, Institute of Molecular Embryology and Genetics, Kumamoto University
* Co-sponsored symposium "Frontiers in Stem Cell Research and Reprogramming"
  Date: October 31-November 1, 2017
  Place: Kyushu University Station-I for Collaborative Research, Auditorium

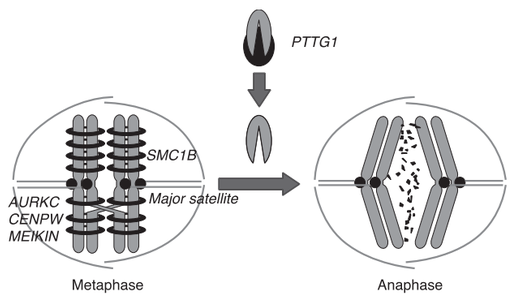
In order to truly understand biological phenomena and disease mechanisms, it is necessary to reconstruct the information network we woven from multiple hierarchical omics data to understand cell strategies (trans-omics research). However, the protocol of trans-omics research does not exist, there are no human resources to realize the foundation (platform). Therefore, in this project, we will develop the world's first common protocol of trans-omics research ("New map of life"), establish research platform and human resource development.

In this project, TMDU medical research intractable disease research institute acquires omics data mainly on three layers of genomics, epigenomics and transcriptomics. Promote creative research that can be a model of trans-omics research by systematically conducting research through cooperation with other three centers. Especially in epigenomics research, we are establishing new hydroxymethylcytosine analysis method and plan to standardize this method and integrate it into the protocol of trans-omics research.



Acquisition of transcriptome data of human early embryo

The epigenetic status of the mammalian genome changes dynamically in pre-implantation stage. This period is also the time when embryo manipulation techniques such as *in vitro* fertilization (IVF) and embryo culture are applied. Therefore, it is possibility that the intervention by embryo manipulation technique has a large influence on epigenetic modification of an individual. In human IVF, it is important to obtain a high pregnancy rate and to be a safe technique. Obtaining gene expression profiles of preimplantation embryos at the early stages of human development is considered to be an important basic data for IVF. On the other hand, the pregnancy rate declines with advanced maternal age. This is believed that the major cause of this reduced pregnancy rate is aneuploidy caused by chromosomal nondisjunction during oocyte meiosis.
Therefore, standard gene expression profiles of human blastocyst stage embryos were obtained from a number of single embryos, and the correlation with various factors was analyzed. As a result, many genes whose expression levels declined with maternal age were identified. Among them, genes important for chromosome segregation during meiosis such as *PTTG1, AURKC, SMC1B and MEIKIN* were included. In addition, transcripts from major satellite repeats constituting the centromere of the chromosome also decreased as the maternal age increased. These results suggest that the epigenetic modification of the oocyte genome varies with the maternal age and may be transmitted to the next generation.



Publications

Kawai K. et al. Parental age and gene expression profiles in individual human blastocysts. Sci Rep. (2018) **8**(1):2380.

Division of Advanced Molecular Medicine

Aim and Scope

The mission of Division of Advanced Molecular Medicine is to conduct basic and applied research on the cause, prevention and treatment of intractable diseases including life-style related diseases, bone diseases, immune diseases, neurological diseases, cardiovascular diseases and cancer. For this purpose, we are actively undertaking a broad spectrum of medical research with an emphasis on cross-disciplinary approaches. Topics of research projects in each Department are as follows:

【Molecular Cell Biology】

- WNK signaling pathway is involved in neural development via GSK3β.
- WDR26 plays a negative role in β-catenin degradation in the Wnt signaling pathway.

【Molecular Neuroscience】

- Calpain-dependent degradation of nucleoporins contributes to motor neuron death in a mouse model of chronic excitotoxicity.
- Glutamate transporter GLAST controls synaptic wrapping by Bergmann glia and ensures proper wiring of Purkinje cells.

【Biodefense Research】

- Discovery of human common monocyte progenitor (cMoP).
- Flexible fate commitment of DC progenitor in tissue microenvironments.
- Intrinsic autophagy is required for the maintenance of intestinal stem cells.

【Bio-informational Pharmacology】

- To facilitate personalized medicine for atrial fibrillation, we found 14 AF-sensitive SNPs in Japanese population, and identified 4 circulating miRNAs as the candidate of biomarker.
- Development of precise optical mapping system with high resolution mapping device and detailed electrophysiological study.
- We reported that gender-specific miRNAs isolated from human heart failure patients were playing in cardiomyocytes, expected as biomarkers for the personalized medicine.

【Stem Cell Regulation】

- Thrombopoietin contributes to the formation and the maintenance of hematopoietic stem cell-containing clusters in the fetal mouse dorsal aorta.
- Dead C6 glioma stem cells (GSCs) were found to increase the expression level of a protumoral marker CD11c in GSC-induced macrophages.

【Structural Biology】

- The crystal structure of RXR with a partial agonist was determined.
- Proteolysis mechanism of Pin-1-derived enzyme was analyzed.

Department of Molecular Cell Biology

Professor Hiroshi Shibuya
Associate Professor Toshiyasu Goto
Assistant Professor Atsushi Sato

Various signaling molecules inducing the cell-growth and differentiation regulate morphogenesis and organogenesis of the vertebrate. The failure of these signal molecules has also been caused with induction of the diseases. Therefore, the elucidation of signal transduction network regulating generation and differentiation is important upon clarifying the mechanism of morphogenesis, organogenesis and diseases. We focus on the signal transduction network regulating the mechanisms of morphogenesis and organogenesis in developmental process.

WNK (with no lysine (K)) kinase family has been recently identified serine/threonin protein kinase family, and is conserved among many species. There are four mammalian WNK family members, and mutations in two of them, WNK1 and WNK4 have been linked to a hereditary form of human hypertension known as Pseudohypoaldosteronism type II (PHAII). WNK1 is also a causative gene of hereditary sensory and autonomic neuropathy type 2A (HSAN2A). Previously, we identified that WNKs phosphorylated and activated SPAK/OSR1 kinases, which in turn regulated various ion co-transporters. We also presented that the malfunction of this regulation caused the similar phenotypes of PHAII in mouse. However, the pathogenesis of HSAS2A is still unknown. Therefore, we started to look for new interacting factor(s) of WNK using model animals, and identified GSK3 β as a candidate.

1. Shaggy is a novel candidate effector of WNK signaling pathway in *Drosophila*

From the screening, we identified *shaggy* (*sgg*) gene as a candidate, which encoded the *Drosophila* homologue of mammalian GSK3 β. In *Drosophila* wing, the overexpression of *WNK* caused ectopic veins. Under *sgg* heterozygous mutant background, the formation of these extra veins by overexpression of *WNK* was suppressed. Furthermore, *WNK* mutant clones led to abdominal developmental defects. Sgg ectopic expression in *WNK* mutant clones rescued the abdominal phenotypes. These results suggest that *sgg* is a novel effector of the WNK signaling pathway.

2. GSK3 β functions as a positive effector downstream of WNK signaling pathway

The expression of GSK3 β induced the expression of *Lhx8* in cultured cells, which is the downstream transcription factor of WNK signaling pathway. Therefore, we examined the epistatic interaction between WNK1 and GSK3 β. The induction of *Lhx8* by overexpression of WNK was suppressed by the knockdown of *GSK3 β*. However, *Lhx8* induction by GSK3 β was not suppressed by the knockdown of both *Wnk1* and *Wnk4*. Furthermore, similar results were obtained in the case of OSR1. These data suggest that the WNK–OSR1–GSK3 β pathway is conserved not only in flies but also in mammals, and that

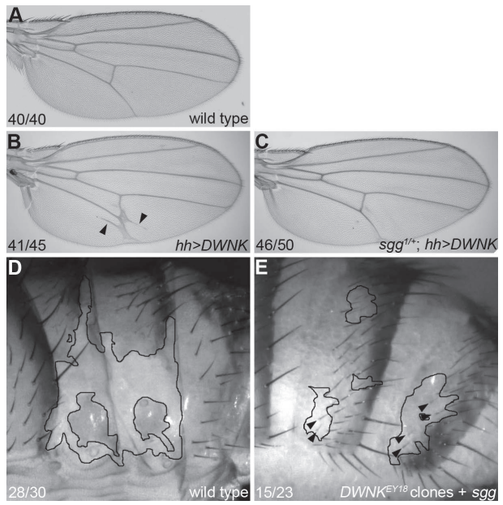


Fig.1 Shaggy is a novel candidate effector of WNK signaling pathway in *Drosophila*

GSK3 β functions as a positive effector downstream of the WNK signaling pathway.

3. GSK3ß is involved in the neural specification

WNK plays an important role in neurite elongation and neural specification through the regulation of *Lhx8* expression. In Neuro2A cells, knockdown of *GSK3 β* caused the shortening of neurites after retinoic acid (RA) stimulation. Knockdown of *GSK3 β* also decreased the expression of *Lhx8* and the choline acetyltransferase gene (*ChAT*; a marker for cholinergic neuron). However, the gene expression of glutamic acid decarboxylase 1 (*Gad1*; a marker for GABAergic neurons) increased. These results were similar to that induced by the knockdown of both *Wnk1* and *Wnk4*. Furthermore, under conditions of both *Wnk1* and *Wnk4* knockdown, the expression of *GSK3 β* partially rescued the elongation of neurites, and *Lhx8* expression; this in turn increased *ChAT* expression and decreased *Gad1* expression. These results suggest that GSK3 β is involved in neural development and functions downstream of the WNK signaling pathway.

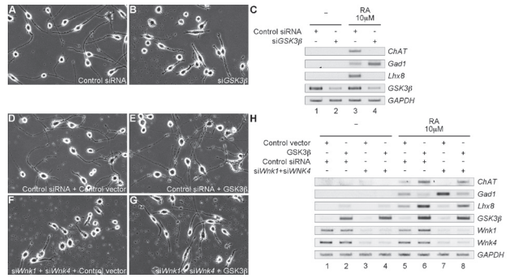


Fig.2 GSK3ß functions as a positive effector downstream of WNK signaling pathway in the neural specification

Publications

Sato, A. and Shibuya, H. (2018). Glycogen synthase kinase 3 β functions as a positive effector in the WNK signaling pathway. **PLoS One** in press.

Department of Molecular Neuroscience

Professor Kohichi Tanaka
Associate Professor Tomomi Aida
Assistant Professor Saeko Ishida
Assistant Professor Yuichi Hiraoka

The final goal of our research is to understand molecular, cellular, and neuronal ensemble mechanisms underlying higher order brain functions including learning and memory. For that purpose, we combine molecular genetics, physiological and behavioral methods. The laboratory also studies the mechanism that underlies neuronal cell death and regeneration.

1. Functions of glutamate transporters in the brain

Glutamate is a major excitatory neurotransmitter and plays an important role in neuronal plasticity and neurotoxicity in the central nervous system. Glutamate transport proteins provide the mechanism by which synaptically released glutamate is inactivated and kept below toxic levels in the extracellular space. By now, five subtypes of high-affinity glutamate transporters have been identified in the mammalian brain. Our lab studies the physiological and pathological roles of glutamate transporter subtypes using subtype-specific knockout mice.

Despite glial glutamate transporter dysfunction leading to excitotoxicity has been documented in many neurological diseases (Figure 1), it remains unclear whether its dysfunction is a primary cause or secondary outcome of neuronal death at disease state. Here we show the combined loss of glial glutamate transporters GLT1 and GLAST in spinal cord caused motor neuronal death and hindlimb paralysis. Further, our novel mutant exhibits the nuclear irregularities and calpain-mediated progressive nuclear pore complex degradation. Our study reveals that glial glutamate transporter dysfunction is sufficient to cause motor neuronal death *in vivo* (Figure 2)

We show that GLAST, a major glutamate transporter in the cerebellar cortex, is essential for synaptic wrapping by Bergmann glia (Figure 3) and synaptic wiring on Purkinje cells (PCs) by parallel fibers (PFs) and climbing fibers (CFs). Without GLAST, monoinnervation of PCs by single strong CFs and segregation of CF and PF territories along PC dendrites cannot develop normally or be maintained. PCs are frequently innervated by additional CF, whereas innervation by main CFs becomes weaker. Ectopic PF synapses appear at proximal dendrites, causing disruption of CF and PF territory segregation along PC dendrites. We conclude that GLAST is indispensable for the establishment of excitatory synaptic wiring to PCs through competition between CFs and between CFs and PFs (Figure 3).

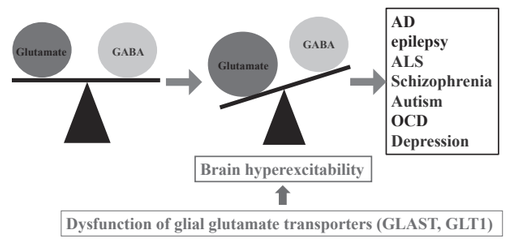


Fig1. Glutamate transporter dysfunction leads to neuropsychiatric diseases

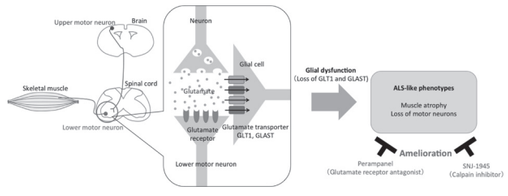


Fig2. Glial glutamate transporters dysfunction induces motor neuron death leading to paralysis

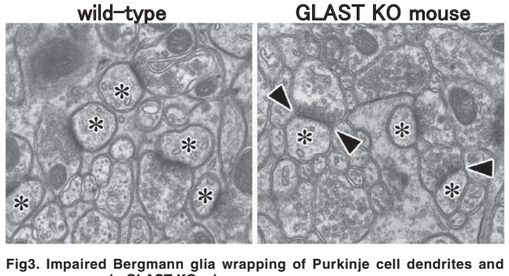


Fig3. Impaired Bergmann glia wrapping of Purkinje cell dendrites and synapses in GLAST KO mice

2. Role of DEPDC5 in the pathogenesis of epilepsy and psychiatric disorder

Epilepsy is one of the most frequent (~1%) neurological disorders characterized by spontaneous and recurrent seizures. However, pharmacoresistance occurs in 30% of the patients. Recently, a role for genetic factors in idiopathic epilepsies, with no identified structural lesion or metabolic cause, is becoming clear. DEP (Dishevelled, Egl-10 and Pleckstrin) domain containing protein 5 (DEPDC5) is a newly identified causative gene for epilepsy (Ishida et al., 2013). DEPDC5 has no transmembrane domain and no homology with known epilepsy genes encode ion channel or transmitter receptor subunits. Its role in epileptogenesis likely differs from the mechanisms known so far. In addition, some individuals also have psychiatric disorder, like autistic features and schizophrenia. This suggests that *DEPDC5* is a common genetic actor in refractory epilepsy and psychosis.

We revealed that Depdc5 inhibits mTORC1 signaling, and Depdc5 KO rats are embryonic lethal (Marsan and Ishida et al., 2016). To avoid the lethality and deeply understand the function of DEPDC5, we conditionally delete Depdc5 in specific brain region or neuronal cells in mice by Cre-loxP system. This year, we have generated Depdc5 floxed mice using CRISPR-Cas9 system. We strongly promote our research with this mouse. Research of DEPDC5 is likely to give new insight into epilepsy and psychosis research.

Publications

[Original papers]

1. Sugiyama, K., Aida, T., Nomura, M., Takayanagi, R., Zeilhofer, HU., Tanaka, K. Calpain-dependent degradation of nucleoporins contributes to motor neuron death in a mouse model of chronic excitotoxicity. *J Neurosci* 37. 8830-8844, 2017
2. Miyazaki, T., Yamasaki, M., Hashimoto, K., Kohda, K., Yuzaki, M., Shimamoto, K., Tanaka, K., Kano, M., Watanabe, M. Glutamate transporter GLAST controls synaptic wrapping by Bergmann glia and ensures proper wiring of Purkinje cells. *Proc. Natl. Acad. Sci. USA.* 114. 7438-7443, 2017.
3. Kubo, K., Deguchi, K., Nagai, T., Ito, Y., Yoshida, K., Endo, T., Benner, S., Shan, W., Kitazawa, A., Aramaki, M., Ishii, K., Shin, M., Matsunaga, Y., Hayashi, K., Kakeyama, M., Tohyama, C., Tanaka, KF., Tanaka, K., Takashima, S., Nakayama, M., Itoh, M., Hirata, Y., Antalffy, B., Armstrong, DD., Yamada, K., Inoue, K., Nakajima, K. Association of impaired neuronal migration with cognitive deficits in extremely preterm infants. *JCI Insight* 2. e88609, 2017.
4. Nakamori, T., Kato, T., Sakagami, H., Tanaka, K., Ohki-Hamazaki, H. Regulation of visual wulst cell responsiveness by imprinting causes stimulus-specific activation of rostral cells. Sci Rep 7. 42927, 2017.

Department of Biodefense Research

Professor	Toshiaki Ohteki, Ph.D.
Junior Associate Professor (fm Jan.16)	Taku Sato, Ph.D.
Assistant Professor (fm June.1)	Masashi Kanayama, Ph.D.
Assistant Professor (til March.31)	Yusuke Nakanishi, Ph.D.
Project Junior Assistant Professor	Jumpei Asano, Ph.D., Mihoko Kajita, Ph.D.
Project Researcher	Shunsuke Kawamura, Ph.D.
Adjunct Lecturer	Nobuyuki Onai, Ph.D.
Research Fellow (SONY) (fm Sept.1)	Yasuharu Yamauchi
Research Technician	Shoko Kuroda, Kisho Shiseki
Research Technician (fm April.1)	Minako Hanabusa
Secretarial Assistant	Hisako Kamioka

Our research projects focus on understanding the dynamic maintenance and transfiguration of homeostasis in the living body. Our goal is to define the homeostasis mechanism under conditions of health and disease. To accomplish this goal, we are trying to clarify the molecular basis of induction and failure of homeostasis by focusing on immune cells in particular **mononuclear phagocytes** (dendritic cells and macrophages), **tissue stem cells**, and their functional interplay in the immunological and non-immunological organs, such as skin and intestine. On the basis of our findings, we will further pursue our research in the hope of developing new rational therapies for prevention and treatment of disease.

1. Research on mononuclear phagocytes

1) Identification of novel sources of mononuclear phagocytes

Mononuclear phagocytes contain monocytes, macrophages and dendritic cells (DCs). In a recent decade, it has been continuing epoch-making discoveries in the field of mononuclear phagocytes and their functions are now beyond classical Immunology and extend to broad life phenomenon, e.g. tissue development/regeneration, wound-healing, and establishment of tumor environments and various inflammatory diseases.

DCs consist of conventional DCs (cDCs) and plasmacytoid DCs (pDCs), both of which play critical regulatory roles in the immune system. cDCs exhibit prominent antigen-presenting ability, whereas pDCs are characterized by their capacity to produce large amounts of type I interferons (IFNs). We have discovered the DC progenitors in the mouse bone marrow, and named common DC progenitors (CDPs) (***Immunity*** 2013; ***Nat Immunol*** 2007). Interestingly, CDPs are divided into 2 subpopulations. One is M-CSF receptor (R)$^{+}$ CDPs mainly producing cDCs, and the other M-CSFR^{-}CDPs producing a large number of pDCs. In addition to CDPs, common monocyte/macrophage progenitors, cMoP, identified in the mouse bone marrow and spleen by other group in 2013.

Based on these achievements in mouse, we have been trying to identify human progenitors of mononuclear phagocytes, and most recently succeeded to identify human cMoP (***Immunity*** 2017). Human cMoP gives rise to only monocytes but not other hematopoietic cells including DCs. Given that monocytes and monocyte-derived macrophages cause a variety of inflammatory disorders, including metabolic syndromes and tumor development, our studies shed light on possible therapeutic applications for infectious diseases, cancers and autoimmune diseases (**Fig. 1**). Collaborations with pharmaceutical company toward the development of therapeutic agents targeting cMoP and monocyte lineage and with Dr. Tomohiro Morio, a Professor of the Department of Pediatrics of TMDU for the pathology clarification of congenital pulmonary alveolar proteinosis (PAP) are currently in progress.

2) Roles of mononuclear phagocytes in the intestinal immune system

Breakdown of the intestinal epithelial layer's barrier function results in the inflow of commensal flora and improper immune responses against the commensal flora, leading to inflammatory bowel disease (IBD) development. Using a mouse dextran sodium sulfate (DSS)–induced colitis model, we showed that commensal Gram-positive bacteria trigger the mobilization of inflammatory monocytes and macrophages into the colon (***Mucosal Immunol*** 2015). TNF-α, a representative cytokine that aggravates colitis and a promising therapeutic target, was predominantly produced by monocytes/macrophages. Among macrophage subpopulations, Ly6c+ macrophages were a major colitogenic subset producing TNF-α. In addition, IFN-γ–Stat1 pathway was required for histone acetylation at the promoter regions of the *Tnf* loci in macrophages, indicating that IFN-γ–dependent epigenetic regulation instructs the development of colitogenic macrophages. Our study may provide new therapeutic targets, e.g. inhibition of acetyl transferase in macrophage, for treating IBD and colon cancer (***Mucosal Immunol*** 2018).

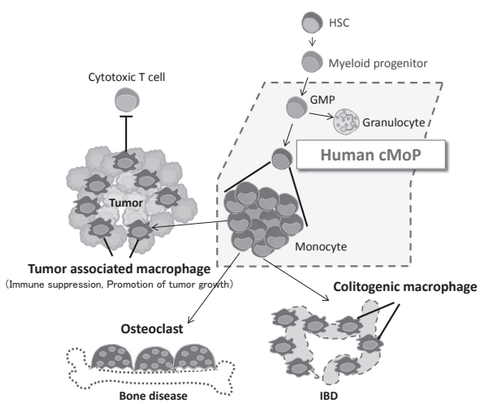


Fig.1 Monocyte-derived macrophage related diseases

Our gut immune system maintains immunological tolerance, not to easily respond to gut commensal bacteria and diet antigens. Generating reporter mice visualizing E2-2, an essential transcription factor for pDC differentiation, we newly found that E2-2^{high} fraction among CDPs strictly gave rise to pDCs in the secondary lymphoid organs when transferred *in vivo*. However, in the small intestine, some of these E2-2^{high} progenitors differentiated into cDCs that produced retinoic acid and induced Foxp3^{+} regulatory T cells. Our findings revealed the commitment and flexibility of E2-2^{high} progenitor differentiation, and implied that pertinent tuning machinery is present in the gut microenvironment (***Int Immunol*** 2017).

2. Research on tissue stem cells

1) Understanding of tissue homeostasis and its breakdown on the basis of immune cell-tissue stem cell interplay

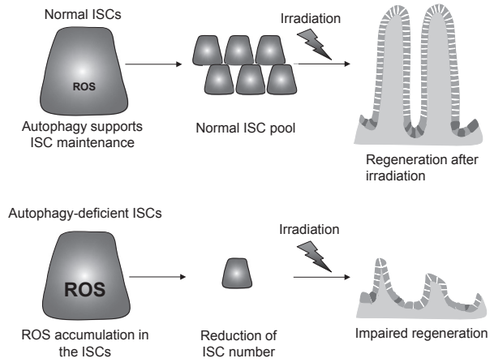


Fig.2 Intrinsic autophagy maintains intestinal stem cells (ISCs)

We found that type I IFNs induce proliferation and exhaustion in hematopoietic stem cells (HSCs), and that interferon regulatory factor-2 (IRF2), a transcriptional suppressor of type I IFN signaling, preserves the self-renewal and multi-lineage differentiation capacity of HSCs (***Nat Med*** 2009). Based on this finding, we show that type I IFN preconditioning, without irradiation or DNA alkylating agents, significantly enhanced the HSC engraftment efficiency in wild type (WT) recipient mice (***Blood*** 2013). Based on these achievements, we have further found that physiological levels of type I IFN signaling also affect other tissue stem cells, e.g. intestinal stem cells (ISCs) and hair follicle stem cells (HFSCs) in collaborations with Drs. Toshiro Sato of Keio University and Emi K. Nishimura of TMDU, respectively (submitted).

In addition, we newly found that intrinsic autophagy is required for the maintenance of intestinal stem cells and for irradiation-induced intestinal regeneration (***Cell Reports*** 2017) (**Fig. 2**).

Publications

1. Kawamura S, Onai N, Miya F, Sato T, Tsunoda T, Kurabayashi K, Yotsumoto S, Kuroda S, Takenaka K, Akashi K and Ohteki T. Identification of a human clonogenic progenitor with strict monocyte differentiation potential - a counterpart of mouse cMoPs. ***Immunity*** 46, 835-48 (2017)
2. Asano J, Sato T, Ichinose S, Kajita M, Onai N, Shimizu S and Ohteki T. Intrinsic autophage in intestinals stem cells is required for their maintenance and for irradiation-induced intestinal regeneration. ***Cell Reports*** 20, 1050-60 (2017)
3. Onai N, Asano J, Kurosaki R, Kuroda S and Ohteki T. Flexible fate commitment of E2-2high common DC progenitors implies tuning in tissue microenvironments. ***Int Immunol*** 29, 443-56 (2017)
4. Nadya NA, Tezuka H, Ohteki T, Matsuda S, Azuma M and Nagai S. PI3K-Akt pathway enhances the differentiation of interleukin-27-induced type 1 regulatory T cells. ***Immunology*** 152, 507-16 (2017).
5. Matsuda K, Okamoto N, Kondo M, Arkwright PD, Karasawa K, Ishizaka S, Yokota S, Matsuda A, Jung K, Oida K, Amagai Y, Jang H, Noda E, Kakinuma R, Yasui K, Kaku U, Mori Y, Onai N, Ohteki T, Tanaka A and Matsuda H. Mast cell hyperactivity underpins the development of oxygen-induced retinopathy. ***J Clin Invest*** 127, 3987-4000 (2017).

[Awards]
Kawamura S, 2017 Medical Research Institute Best Article Award

[Personnel Changes]
Moving out:
Nakanishi Y, Assistant Professor of College of Bioresource Sciences, Nihon University, Kanagawa, Japan.

Moving in:
Kanayama M, Assistant Professor from Duke University, USA.
Sato T, Junior Associate Professor from JST PRESTO Researcher, Saitama, Japan.

Department of Bio-informational Pharmacology

Professor Tetsushi Furukawa, M.D., Ph.D.
Associate Professor Jun Takeuchi, Ph. D.
Assistant Professor Kensuke Ihara, M.D., Ph.D.

This laboratory focuses on understanding pathogenesis of intractable and common cardiovascular diseases using multidisciplinary approach (patch-clamp, cell biology, optical recording, genetic analysis, and computational analysis). Our ultimate goal is to improve diagnosis and management of intractable and common cardiovascular diseases.

1. Personalized medicine for atrial fibrillation (AF)

Combining discovery panel and replication panel, we performed GWAS in 113,00 AF cases and 153,676 controls in Japanese population, and identified total 14 AF-sensitive SNPs. We calculated weighed genetic risk score (wGRS) and divided them into quartiles; the highest quartile group (top 25%) had 7.58-fold higher incidence of AF compared to the lowest quartile group (bottom 25%) (integrated odds ratio) (***Nat. Genet***. 2017;70:180-184).

In order to carry out personalized medicine involving medical intervention, we require odds ratio up to around 50. Thus, to raise odds ratio from less than 10 to about 50 is the bottle-neck to materialize personalized medicine. Since most SNPs with high odds ratio have been identified through 3 rounds of GWAS, it is impossible by increasing sample numbers for genetic analysis to overcome this bottle-neck. Then, we searched for AF-sensitive biomarkers, and identified 4 AF biomarker candidates (see highlight).

2. Electrophysiological Assessment of Murine heart with High-Resolution Optical Mapping

Conventional optical mapping of murine heart, especially of its atria, has some critical problems due to its small size. To overcome them, we developed the novel electrophysiological assessment method for elucidating the underlying mechanism of arrhythmogenesis using murine heart by combining high spatial and temporal resolution optical mapping system and precise electrophysiological study (*J. Vis. Exp* 2018 in press). This novel method will contribute to assessing the onset and maintenance mechanism of arrhythmias precisely in various mouse models.

3. Sex specific mRNAs/miRNAs expression in human/murine heart diseases

Sexual dimorphisms in various heart diseases; ex ICM, MI and DCM are well known. However, the questions why these diseases onset in men/males are higher than in women/females remain unsolved. Here, we show that the transcriptional profiles of mRNAs and miRNAs between men/males and women/females from left ventricles in heart development, their failures and MI models. In this profile, mRNA and miRNA transcriptome of normal and disease heart show significant sex differences, which might impact the cardiac homeostasis (Tsuji et al., PLoS One 2017). Especially, we identified 2 miRNAs; miR2861, miR-139-5p, which have unique expression patters during heart development and its disease by *in silico* analysis and GO analysis. Together this study provides the first comprehensive picture of the genome-wide program underlying the heart sexual dimorphisms, laying the foundation for gender specific treatment strategies.

Highlight

AF-sensitive biomarker candidates miRNA

We compared circulating miRNA between serum from 100 AF patients and those from 100 controls with miRNA array. We also compared circulating miRNA between AF mice and non-AF mice in 2 mouse AF models, a pressure-overload model and a high-fat-diet model. We found 4 circulation miRNAs (miR-99a-5p, miR-192-5p, miR-214-3p, miR-342-5p), which showed significant difference between AF and non-AF both in human and mouse. Combining 4 miRNAs, we could segregate AF from non-AF with 76% sensitivity and 80% selectivity (Figure).

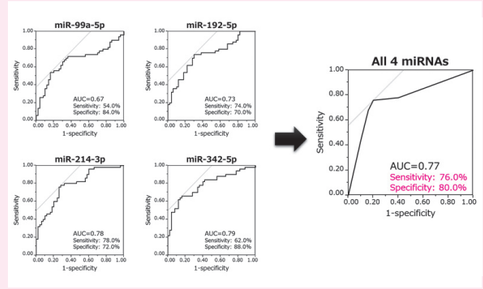


Figure. AF-sensitive biomarker candidates four miRNAs
We analyzed the precision of biomarker candidates using ROC analysis. Combining 4 miRNAs, we achieved 76% sensitivity and 80% specificity.

Publications

[original articles]

1. Kuroda Y, Yuasa S, Watanabe Y, Ito S, Egashira T, Seki T, Hattori T, Ohno S, Kodaira M, Suzuki T, Hashimoto H, Okata S, Tanaka A, Aizawa Y, Murata M, Aiba T, Makita N, Furukawa T, Shimizu W, Kodama I, Ogawa S, Kokubun N, Horigome H, Horie M, Kamiya K, Fukuda K. Flecainide ameliorates arrhythmogenicity through NCX flux in Andersen-Tawil syndrome-iPS cell-derived cardiomyocytes. ***Biochem. Biophys. Rep.*** 2017;9:245-256.
2. Liu L, Ebana Y, Nitta J, Takahashi Y, Miyazaki S, Tanaka T, Komura M, Isobe M, Furukawa T. Genetic variants associated with susceptibility to atrial fibrillation in Japanese population. ***Can. J. Cardiol.*** 2017;33:443-449.
3. Zaw KTT, Sato N, Ikeda S, Thu KS, Mieno MN, Arai T, Mori S, Furukawa T, Sasano T, Sawabe M, Tanaka M, Muramatsu M. Association of ZFHX3 gene variation with atrial fibrillation, cerebral infarction, and lung thromboembolism: An autopsy study. ***J. Cardiol.*** 2017;70:180-184.
4. Low SK, Takahashi A, Ebana Y, Ozaki K, Christophersen IE, Ellinor PT; AFGen Consortium., Ogishima S, Yamamoto M, Satoh M, Sasaki M, Yamaji T, Iwasaki M, Tsugane S, Tanaka K, Naito M, Wakai K, Tanaka H, Furukawa T, Kubo M, Ito K, Kamatani Y, Tanaka T. Identification of six new genetic loci associated with atrial fibrillation in the Japanese population. ***Nat. Genet.*** 2017;49:953-958.
5. Ebana Y, Ozaki K, Liu L, Hachiya H, Hirao K, Isobe M, Kubo M, Tanaka T, Furukawa T. Clinical utility and functional analysis of variants in atrial fibrillation-associated locus 4q25. ***J. Cardiol.*** 2017;70:366-373.
6. Miyazaki S, Ebana Y, Liu L, Nakamura H, Hachiya H, Taniguchi H, Takagi T, Kajiyama T, Watanabe T, Igarashi M, Kusa S, Niida T, Iesaka Y, Furukawa T. Chromosome 4q25 variants and recurrence after second-generation cryoballoon ablation in patients with paroxysmal atrial fibrillation. ***Int. J. Cardiol.*** 2017;244:151-157.
7. Ebana Y, Nitta J, Takahashi Y, Miyazaki S, Suzuki M, Liu L, Hirao K, Kanda E, Isobe M, Furukawa T. Association of the clinical and genetic factors with superior vena cava arrhythmogenicity in atrial fibrillation. ***Circ. J.*** 2017;82:71-77.
8. Min Li, Kanda Y, Ashihara T, Sasano T, Nakai Y, Kodama M, Hayashi E, Sekino Y, Furukawa T, Kurokawa J. Overexpression of KCNJ2 in induced pluripotent stem cell-derived cardiomyocytes for the assessment of QT-prolonging drugs. ***J. Pharmacol. Sci.*** 2017;134:75-85.
9. Tsuji M, Kawasaki T, Matsuda T, Arai T, Gojo S, Takeuchi JK. Sexual dimorphisms of mRNA and miRNA in human/murine heart disease. ***PLoS One*** 2017;12:e0177988.

Department of Stem Cell Regulation

Professor	Tetsuya TAGA
Associate Professor	Ikuo NOBUHISA
Assistant Professor	Kouichi TABU
Adjunct Lecturer	Ryoichiro KAGEYAMA, Taichi KASHIWAGI
Collaborative Researcher	Tetsushi KAGAWA, Norihisa Bizen
Administrative Assistant	Kazuko INOUE
Graduate Student	Wenqian WANG, Yoshitaka MUROTA, Kiyoka SAITO, Alapati AIMAITIJIANG, Yuki YOKOI, Haruka EISHI, Satomi TAKAHASHI, Hiroaki MATSUNAGA, Ruriko TATSUMI, Koya AZUMA, Naoki IIZUKA, Risa OKUNO, Yusaku MIHANA

Research Outline

Our research has been conducted to elucidate the mechanisms by which stem cells are regulated. The major focus has been on neural stem cells, hematopoietic stem cells, and cancer stem cells. The study is aimed to understand development, maintenance, and regeneration of the central nervous system and the hematopoietic system, and to obtain a clue to tackle the problem of cancer recurrence. The projects performed in 2017 are categorized into three groups: 1. Understanding of molecular basis for self-renewal and fate determination of neural stem cells, 2. Characterization of fetal hematopoiesis, and 3. Characterization of cancer stem cells and their niche.

Research Projects

1. Contribution of thrombopoietin to formation and maintenance of hematopoietic progenitor cell-containing cell clusters in the aorta-gonad-mesonephros region

In midgestation mouse embryo, hematopoietic cell clusters containing hematopoietic stem/progenitor cells first appear in the lumen of the dorsal aorta of the aorta-gonad-mesonephros (AGM) region. We have previously reported that forced expression of the Sox17 transcription factor in CD45^{low}c-Kit^{high} AGM cells, which are one of the components of hematopoietic cell clusters, and subsequent coculture with OP9 stromal cells in the presence of three cytokines, stem cell factor (SCF), interleukin-3 (IL-3) and thrombopoietin (TPO), leads to the formation and maintenance of hematopoietic cell clusters containing cells at an undifferentiated state in vitro. We investigated the role of each cytokine among SCF, IL-3, and TPO in the formation of hematopoietic cell clusters. We cultured Sox17-transduced cells with either of the all the 7 possible combinations of the three cytokines. The size and the number of Sox17-transduced cell clusters in the presence of TPO, either alone or in combinations, were comparable to that observed with the three cytokines added simultaneously. Next, we examined expression of TPO receptor, c-Mpl, in Sox17-transduced hematopoietic cell clusters. Expression of c-Mpl was highly maintained in the hematopoietic cell clusters. In addition, the CD45^{low}c-Kit^{high} cells expressed the highest level of the c-Mpl among the populations of Sox17-transduced cells classified by the expression profiles of CD45 and c-Kit. Moreover, we analyzed the c-Mpl expression in the fetal mouse AGM region by immunohistology. The c-Mpl protein was highly expressed in hematopoietic cell clusters in comparison with endothelial cells of dorsal aorta. These results suggest that TPO contributes to formation and the maintenance of hematopoietic cell clusters in the AGM region (Figure 1).

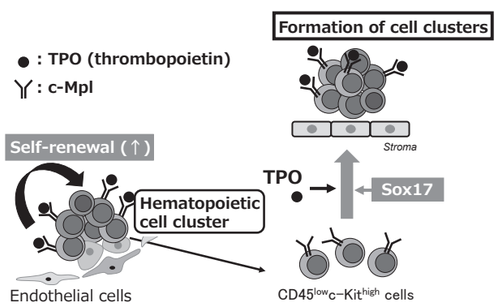


Figure 1: Contribution of the TPO/c-Mpl signaling pathway to the formation and the maintenance of hematopoietic cell clusters

2. Elucidation of self-organized cancer stem cells niche

"Cancer stem cells" (CSCs), a small subset of tumor cells, are characterized by chemo/radio-resistance and have the ability to reconstitute original tumors. Therefore, CSCs are a key driver of tumor relapse and have been proposed as a promising target to eradicate cancers. As we published in 2004, C6 glioma cell line contains a subpopulation of CSCs, which is enriched in the "side population (SP)" by Hoechst 33342 staining and FACS analysis. Recently we have reported that CSCs have a self-expanding strategy that organizes their own niche composed of vascular and immune cells. In this year, we aimed to establish new therapeutic strategies especially by focusing on phagocytosing activity of tumor-associated macrophages (TAMs) developed by CSCs. When incubated with SP-derived dead cells, SP-induced protumoral macrophages (SP-Mφs) phagocytosed them and exhibited much larger cell body, and the number of Mφs expressing a protumoral marker CD11c was significantly increased, suggesting that glioma CSCs may contribute to tumor progression by affecting TAMs through their own cell death. Since iron-storing Mφs were observed in SP-transplanted tumors and SP cells express a receptor for iron-carrier transferrin, we next hypothesized that CSCs might replenish iron by exploiting TAMs that phagocytose hemorrhaged erythrocytes and recycle iron. SP-Mφs were confirmed to contact with aged erythrocytes and iron chelation in vitro was found to remarkably suppress the growth of SP cells. SP-Mφs also increase the expression of FPN gene encoding an iron exporter by approximately 9.6-fold compared with bone marrow monocytes, suggesting that glioma CSCs-secreted factor(s) confer an ability of supplying iron to their induced TAMs. Finally, database analysis of gene expression in glioma patients demonstrated that the expression of FPN and a TAM marker CD204 genes in glioblastoma (WHO grade IV) patients is positively correlated with each other, and astrocytoma (WHO grade II and III) patients with higher expression of FPN gene exhibit relatively poorer survival than those with lower expression. These findings could provide clues to understand the pathobiology of CSC-organized niche and to establish effective therapeutic strategies for cancer eradication (Figure 2).

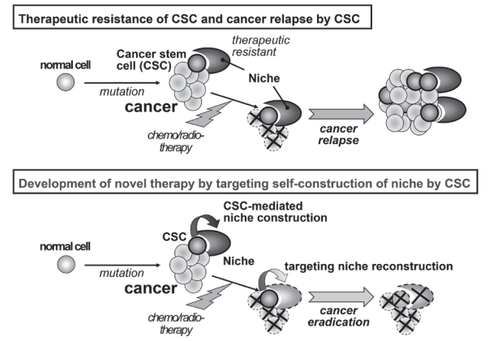


Figure 2: The concept of CSC-mediated niche construction and cancer eradication

Publications

[Original Article]

1. Wang W, Tabu K, Hagiya Y, Sugiyama Y, Kokubu Y, Murota Y, Ogura SI, Taga T. Enhancement of 5-aminolevulinic acid-based fluorescence detection of side population-defined glioma stem cells by iron chelation. Scientific Reports, 7:42070, 2017.
2. Harada K, Nobuhisa I, Anani M, Saito K, Taga T. Thrombopoietin contributes to the formation and the maintenance of hematopoietic progenitor-containing cell clusters in the aorta-gonad-mesonephros region. Cytokine, 95:35-42, 2017.

Department of Structural Biology

Professor Nobutoshi Ito
Associate Professor Teikichi Ikura
Assistant Professor Nobutaka Numoto

The advance of genome science and proteomic analysis has produced a large amount of information about the primary structure of proteins and their spatial and temporal distributions. On the other hand, most of the proteins only function when they take certain three dimensional structures. As obviously seen in so-called prion diseases, proteins which are chemically correct but structurally incorrect not only fail to function properly but also can harm cells. Our laboratory aims to understand the function of biological macromolecules at atomic level through structure analysis and other methods of physical chemistry, in the hope that accumulation of such knowledge will eventually lead to development of drugs. We are also involved in providing database of such structural data to scientists through the activities of Protein Data Bank Japan.

1. Structural analysis of the complex of the nuclear receptor and a partial agonist

The nuclear receptors bind lipophilic small molecules called hormones, and regulate transcription of genes. The functions of nuclear receptors and their specific ligands are closely involved in the onset and treatment of cancer, autoimmune diseases, so that ligand molecules that regulate signaling of nuclear receptors are actively studied to apply for pharmaceuticals. Retinoid X receptor (RXR) has been studied as a major nuclear receptor, and various agonists, antagonists, and partial agonists have been developed followed by the structural studies of its complexes. However, the detailed model of the complex of RXR and partial agonists, which show intermediate activities, is hardly elucidated. It is important to understand the molecular mechanism of the activation by partial agonists from the viewpoint of compatibility between reduction of side effects and control of activity. In this study, we have determined the crystal structure of the complex of the ligand binding domain of human RXR and a novel synthesized partial agonist CBt-PMN (Kakuta et al, ACS Med Chem Lett., 3, 427, 2012).

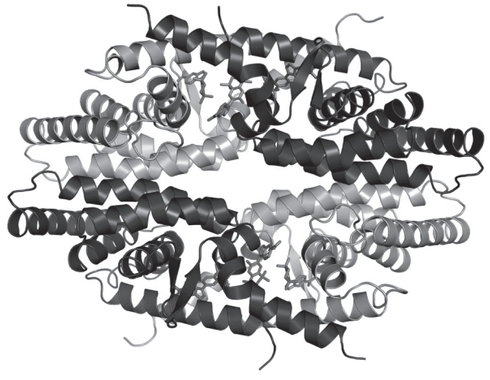


Fig.1 Crystal structure of the complex of the ligand binding domain of RXR and the partial agonist CBt-PMN.

The structure reveals that RXR forms a tetramer and six molecules of CBt-PMN are bound to the tetramer (Fig. 1). Four molecules are bound near the large hydrophobic pocket, which is known as a ligand binding site of nuclear receptors, in each monomer of RXR. Interestingly, the binding mode of these four molecules can be divided into two forms. The remaining two molecules are bound to the interface of the subunits. These findings are distinctly different from the known agonist-binding forms. In particular, a remarkable difference in the orientation of the hydrophobic-rich portion of CBt-PMN is observed. In addition, the structure of each monomer of RXR is close to that of apo-RXR to which no ligand molecule is bound. The structure obtained in this study reveals a novel binding mode of the partial agonist of RXR, and gives important structural basis for elucidating the molecular mechanism of the partial agonist activity.

This work is performed in collaboration with Professor Tokiwa of Rikkyo University.

2. Catalytic mechanism of a protease derived from Pin1

A peptidyl-prolyl isomerase, Pin1, functions as a switch in the cell cycle, a regulator for the Alzheimer's disease, and so on, but its catalytic mechanism is still unknown. We were investigating the mechanism by a comprehensive site-directed mutagenesis for the residue C113 which was essential to the catalysis. In this study, we found that several mutations like C113A deactivated the catalytic activity but created a proteolytic activity, that was, a single mutation converted the isomerase activity of Pin1 into the proteolytic activity (Fig. 2). Since then, we have been tackling a project to elucidate the catalytic mechanism of the novel protease derived from Pin1.

So far at least, we have elucidated that this protease recognizes a proline residue and cleaves the peptide bond of the amino acid four residue earlier by mass spectrometry and site-directed mutagenesis analysis. The catalytic site is indicated to be typical triad in serine protease.

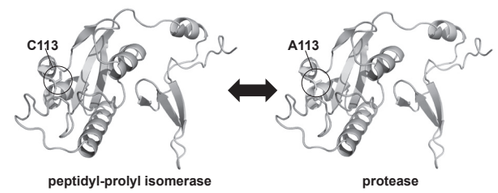


Fig.2 An alanine substitution at C113 converts the isomerase activity of Pin1 into the proteolytic activity.

Further investigation, however, suggests that it has another catalytic site which cleaves the bond in a nonspecific manner. Our data also suggests that wild-type Pin1 potentially possesses proteolytic activity and its activity is expressed under a specific condition.

3. Improvement in Protein Data Bank

The advance of structural biology with X-ray crystallography and NMR has generated a huge amount of data such as atomic coordinates. The increase is expected to continue with even more rapidly by the advent of structure genomics. The vast information is now a precious resource of wide range of science such as bioinformatics. Ever since 1970's, Protein Data Bank (PDB) has been acting as the main and single international archive of the structural data of biological macromolecules. Currently wwPDB, a consortium of Research Collaboratory of Structural Bioinformatics (RCSB), European Bioinformatics Institute (EBI) and Protein Data Bank Japan (PDBj) maintains it. We, as a member of PDBj, contribute to the continuous effort by wwPDB to improve PDB.

Publications

1. Satomi Inaba, Nobutaka Numoto, Shuhei Ogawa, Hisayuki Morii, Teikichi Ikura, Ryo Abe, Nobutoshi Ito, Masayuki Oda. Crystal Structures and Thermodynamic Analysis Reveal Distinct Mechanisms of CD28 Phosphopeptide Binding to the Src Homology 2 (SH2) Domains of Three Adaptor Proteins. J. Biol. Chem.. 2017.01; 292(3); 1052-1060
2. Yurina Miyashita, Eiji Ohmae, Teikichi Ikura, Kaoru Nakasone, Katsuo Katayanagi. Halophilic mechanism of the enzymatic function of a moderately halophilic dihydrofolate reductase from Haloarcula japonica strain TR-1. Extremophiles. 2017.05; 21(3); 591-602

Frontier Research Unit
Laboratory of Oxygen Biology

Associate Professor Koh Nakayama, Ph.D.
Assistant Professor Ryo Yonashiro, Ph.D.

Aim of the Research

Study in our laboratory aims to understand how changes in the ambient oxygen concentration affect our body function. Oxygen is required for the efficient energy production in mitochondria. Furthermore, recent studies revealed the role of oxygen, particularly a hypoxic condition, involved in developmental processes, tumorigenesis, and stem cell function. Our goal is to understand the molecular mechanism of hypoxic response, and establish new tools for cancer therapy and regenerative medicine.

Research Projects

1. Metabolic regulation mediated by hypoxia complex

Hypoxia-Inducible Factor (HIF)-α is a transcription factor which plays a central role during hypoxic response. HIF-α is actively degraded during normoxic condition, whereas it is stabilized and activated under hypoxic conditions. PHD hydroxylates and regulates the expression of HIF-α. There are three PHDs in mammals; namely PHD1, 2, and 3. We have previously identified that PHD3 forms a complex under hypoxic conditions (Figure). This complex serves to stabilize HIF-α by inhibiting the access of PHD3 to HIF-α.

Moreover, we recently identified that pyruvate dehydrogenase (PDH) is included in this complex (Figure). PDH is an enzyme which converts pyruvate into acetyl CoA, and plays a key role in energy metabolism. We have demonstrated that PDH interacts with PHD3 under hypoxic condition. In *PHD3*$^{-/-}$ cells, PDH activity is significantly decreased, suggesting that PHD3 positively regulates PDH activity by directly interacting with it. Cancer cells are known to exhibit glycolytic metabolism; however, the molecular mechanism of how such metabolism is formed has not been elucidated yet. We aim to understand the mechanism by focusing on the PHD3-PDH interaction mediated by the hypoxia complex.

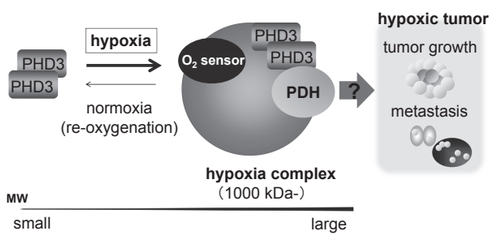


Figure Cellular metabolism regulated by Hypoxia Complex

2. Molecular machinery of aerobic glycolysis in cancer cells

We further analyzed the role of PDH in hypoxia and identified that PDH-E1β subunit is downregulated during chronic hypoxia. This downregulation is sustained upon reoxygenation, therefore causing glycolytic metabolism in breast cancer cells even under normoxic condition. Furthermore, knockdown of PDH-E1β in cancer cells caused a Warburg effect-like metabolism, which also points to the importance of PDH-E1β downregulation on inducing glycolytic metabolism in cancer cells. Finally, PDH-E1β-KD cells formed smaller tumor than the control cells in nude mice, indicating that sustained glycolytic metabolism is not sufficient to promote tumor growth, and implies a possibility of cancer cells also utilizing oxidative phosphorylation for their efficient energy production.

Publications

1. Yonashiro R., Eguchi K., Wake M., Takeda N., and Nakayama K.* Pyruvate dehydrogenase PDH-E1β is downregulated under prolonged hypoxic conditions and controls tumor progression by altering the metabolic status of cancer cells. ***Cancer Res.*** **(2018)** *in press*

2. Gudla PR., Nakayama K., Pegoraro G., Misteli T. SpotLearn: Convolutional Neural Network for Detection of Fluorescence In Situ Hybridization (FISH) Signals in High-Throughput Imaging Approaches. ***Cold Spring Harb Symp Quant Biol.*** **(2017)** *in press*

Tenure Track Research Unit
Department of Cellular and Molecular Medicine

Associate professor Yumiko Oishi MD., PhD.
Assistant professor Sumio Hayakawa, PhD.
Shinichiro Hayashi, PhD. (~June 2017)
Research Technician Yukiko Hoshino

Research outline

Cardiovascular disease, as a consequent of the obesity related metabolic syndrome, remains a significant cause of morbidity and mortality in industrialized societies. A major effort of our laboratory has been to investigate the molecular mechanism of metabolic syndrome from the viewpoint of transcriptional regulation. We focus on macrophage and skeletal muscle as major players in those contexts. The long-term goals of our current study are to elucidate: 1) the mechanism of the link between cellular metabolism and immune response in macrophage 2) the mechanism of chronic inflammation leads to metabolic syndrome, and 3) the mechanism responsible for pathogenesis of sarcopenia and skeletal muscle regeneration.

Research Project

1. Mechanisms of Coordinated regulation of inflammatory response and lipid homeostasis in macrophage

Chronic low-grade inflammation has been recognized as a key contributing factor in the onset and progression of metabolic syndrome and atherosclerosis. As a multifunctional effector cell, macrophage play pivotal roles in both the enhancement and resolution of this inflammatory process. By utilizing molecular biology technique, lipidomics and bioinformatics, we found that the lipid homeostasis is coordinately regulated with inflammatory response in macrophage. TLR4 activation rapidly, and transiently inhibits Liver X receptor (LXR) signaling, and subsequently activates Sterol regulatory element-binding protein (SREBP). In the late phase of inflammation, LXR and SREBP work together to increase anti-inflammatory fatty acid synthesis, necessary for a resolution of inflammation. Thus, transcriptional/signaling network involving LXR and SREBP play a pivotal role in the regulation of lipid homeostasis and cellular function. By elucidating the crosstalk between cellar function and metabolism, we would be able to accumulate beneficial knowledge to develop novel therapeutic strategy targeting macrophages for the prevention and treatment of metabolic syndrome (Fig.1).

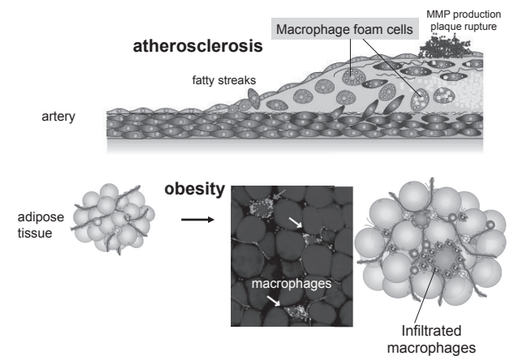


Fig.1

2. Mechanism of skeletal muscle degeneration

Skeletal muscle consume ~40% of total energy, playing a key role for the pathogenesis of metabolic syndrome. Sarcopenia is the degenerative loss of skeletal muscle mass, quality and strength associated with aging. Although the causes and mechanisms of sarcopenia still remains unclear, one of the hypotheses is dysfunction of satellite cells, muscle stem cells in muscle. We identified KLF5 as a novel factor that play a pivotal role in skeletal muscle degeneration. Klf5 is a Zinc-finger transcription factor involved in the self-renewal and proliferation of embryonic stem cell and cancer stem cell. KLF5 is transiently induced in the myogenesis, plays critical role for muscle degeneration and repair. Although Klf5 expression is low in quiescent satellite cells, its expression is highly induced with age. Now we are testing the hypothesis whether the dysregulation of Klf5 causes a dysfunction of satellite cells.

Publications

1. Oishi Y, Spann, NJ, Link VM, Muse ED, Strid T, Edillor C, Kolar MJ, Matsuzaka T, Hayakawa S, Tao J, Kaikkonen M, Lam MT, Manabe I, Shimano H, Saghatelian A and Glass CK. SREBP1 contributes to resolution of pro-inflammatory TLR4 signaling by reprogramming fatty acid metabolism. ***Cell Metab***, 25:412-427, 2017.
2. Oishi Y, Hayashi S, Isagawa T, Oshima M, Iwama A, Shimba S, Okamura H and Manabe I. Bmal1 regulates inflammatory responses in macrophages by modulating enhancer RNA transcription. ***Sci Rep*** 7:7086, 2017.

Frontier Laboratory: Skeletal Molecular Pharmacology

Associate Professor　Yoichi Ezura, MD, PhD

Summary

To elucidate the molecular regulation of the cellular responses in organs and tissues involved in the calcium regulatory system, we focus on contributing to the establishment of the prevention and treatment of skeletal disorders.

Research Project

1. Lgr4 expression is suppressed by hydrogen peroxide in osteoblasts.

(***J Cell Physiol***, 2017, **Pawaputanon Na Mahasarakham et al.**)

2. Lysosomal channel protein TPC2 suppresses osteoclast differentiation under the low-magnesium condition

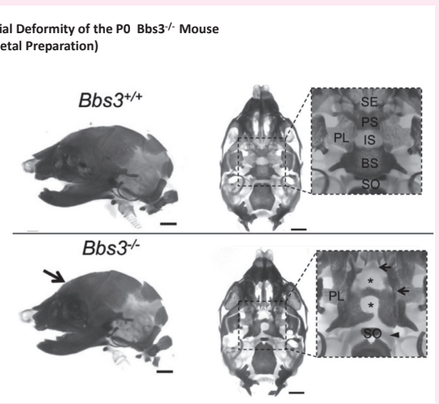
(***J Biol Chem*** 2017, **Dr. Notomi et al.**)

Highlight

Bardet-Biedl Syndrome 3 in the development of cranial base midline structures.

- Bbs3$^{-/-}$ mice manifested developmental craniofacial dysmorphism (CD) : At birth, cranium was "dome-shaped" and mid-face was hypoplastic.
- At E18.5, partially mineralized cranial-base was longitudinally short but laterally expanded possibly due to the impaired fusion. At E14.5, cartilaginous primordium was already hypomorphic.
- Mechanistically, at E12.5, the ecto-mesenchymal condensation, associated with intense Bbs3 expression was impaired at midline.
- Bbs3-silenced ATDC5 cells were defective in migration, despite canonical Hh-signaling was intact.
- These results implied the involvement of impaired migration of Bbs3$^{-/-}$ neural crest cells toward Shh-gradient independent of classical Shh-pathway.

(***Bone***, 2017, **Kawasaki et al.**)



Publications

[Original articles]

1. Lin W, Izu Y, Smriti A, Kawasaki M, Pawaputanon C, Böttcher RT, Costell M, Moriyama K, Noda M, Ezura Y. Profilin1 is expressed in osteocytes and regulates cell shape and migration. J Cell Physiol. 2018, 233(1):259-268.
2. Notomi T, Kuno M, Hiyama A, Nozaki T, Ohura K, Ezura Y, Noda M. Role of lysosomal channel protein TPC2 in osteoclast differentiation and bone remodeling under normal and low-magnesium conditions. J Biol Chem. 2017, 292(51):20998-21010.
3. Katsumura S, Izu Y, Yamada T, Griendling K, Harada K, Noda M, Ezura Y. FGF Suppresses Poldip2 Expression in Osteoblasts. J Cell Biochem. 2017, 118(7):1670-1677.
4. Pawaputanon Na Mahasarakham C, Izu Y, Nishimori K, Izumi Y, Noda M, Ezura Y. Lgr4 Expression in Osteoblastic Cells Is Suppressed by Hydrogen Peroxide Treatment. J Cell Physiol. 2017, 232(7):1761-1766.
5. Kawasaki M, Izu Y, Hayata T, Ideno H, Nifuji A, Sheffield VC, Ezura Y, Noda M. Bardet-Biedl syndrome 3 regulates the development of cranial base midline structures. Bone. 2017, 101:179-190.

Division of Pathophysiology

【Aim and Scope】

Research purpose of the Division of Pathophysiology is to understand the fundamental mechanisms underlying biological phenomena and their abnormalities in the disease conditions including intractable diseases and to develop new diagnostic or therapeutic tools for various diseases of which curative treatment is difficult. Topics of research projects in each Department are as follows;

【Neuropathology】

- Discovery of a common pathology across Alzheimer's disease and frontotemporal lobar degeneration.
- Developmental YAPdeltaC determines adult SCA1 pathology.

【Pathological Cell Biology】

- Identification of Dram1 as an execution molecule for alternative autophagy.
- Autophagic cell death compensates apoptosis through the process of development.

【Developmental and Regenerative Biology】

- Molecule's role in maintaining liver size and function revealed
- Signaling pathway controls body clock and movement in adult mice

【Stem Cell Biology】

- Elucidation of the mechanisms of epidermal aging

【Immunology】

- Elucidation of the crucial role of reactive oxygen species in B lymphocyte activation.
- Development of the method to identify glycan-ligands on the same cell (cis-ligands) of the inhibitory B cell co-receptor CD22/Siglec-2 that regulate functional activity of CD22, and identification of a novel CD22 cis-ligand.

【Molecular Pathogenesis】

- Transgenic mouse line overexpressing MKL1 in macrophages is a model for inflammatory bowel disease.
- Overexpression of PP1M-inhibitory molecule M21 in mice results in hypertrophic cardiomyopathy followed by dilated cardiomyopathy.

Department of Neuropathology

Professor	Hitoshi Okazawa
Associate Professor	Kazuhiko Tagawa
Project Lecturer/Part-time Lecturer	Haruhisa Inoue, Masaki Sone, Toshiki Uchihara
Assistant professor	Kyota Fujita
Project Assistant professor	Xigui Chen, Hidenori Homma, Emiko Yamanishi

Outline

Following studies have been intensively carried out in our laboratory with various techniques including molecular biology, cell biology, biochemistry, Drosophila models, and mice models.

1) Investigation of molecular pathologies of neurodegenerative disease.
2) Development of new therapies for neurodegeneration.
3) Development of new seed drug for mental retardation.
4) Investigation of molecular function of Oct3/4.

This year's progress

1. Preclinical pathology during development affects the post-onset adulthood symptoms in spinocerebellar ataxia type 1 (Fujita K, et al, Nat Commun. 2017)

Spinocerebellar ataxia type1 (SCA1) is one of the major models of neurodegenerative disease. At the same as other neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS), disease modifying therapy for SCA1 has not developed. Although a lot of knowledge for pathology caused by mutation has also been accumulated, it has not been clear neither when it occurs, what it is, nor how to treat it. For instance, in context of therapeutic strategies of AD, there were many clinical investigations to remove amyloid-beta, however, clinical symptoms did not improve or stop the progression despite elimination of amyloid-beta. This fact tells us that it is quite important to uncover that when irreversible pathology of neurodegenerative diseases occurs and what the pathology is.

In our previous study, we studied about the effect of stage-specific expression of YAPdeltaC to symptoms of SCA1 model mice (Ataxin-1 knock-in mice) and their survival time using our newly developed Tet-ON YAPdeltaC system in Atxn1-KI mice. As a result, expression of YAPdeltaC from embryo stage to 8 weeks after birth made significant improvement of symptoms and survival duration, however, almost no effect found with expression of YAPdeltaC after 8 weeks.

Moreover, we revealed that YAPdeltaC works collaboratively with normal Ataxin1 as transcriptional coactivator enhancing RORalpha, that plays a role of regulation of gene expression in cerebellar neurons at developmental stage. Because mutant Ataxin1 deprives YAPdeltaC from RORalpha, it inhibits expressions of genes necessary for maturation of cerebellar neurons. We also found that forcible expression of YAPdeltaC increases its binding of RORalpha and that makes a recovery of gene expressions required to mature cerebellar neurons.

Clinical studies to examine candidate drugs by starting the clinical trial before onset are now undergoing in USA, and are also planned in Japan. Our study showed that recovery of gene expressions required for maturation of cerebellar neurons in developmental stage improves clinical symptoms of SCA1 model mice in adulthood. Because genetic diagnosis can be available for SCA1 patients, it could be possible to develop prophylactic or disease modifying therapy in the future by increasing amount of YAPdeltaC at preclinical stage. This study presented a great example that preclinical therapy was needed for neurodegenerative diseases commonly, not only for AD. At the same time, it has great significance that revealed its therapeutic target molecule.

2. Insights into familial middle-age dementia suggest new avenues for treatment (Fujita K, et al, Nat Commun. 2018)

Frontotemporal lobar degeneration (FTLD) is one of the most common causes of early-onset dementia, and can lead to personality changes, impaired speaking, and motor dysfunction. While most cases occur sporadically, several inherited forms of FTLD have been linked to genetic mutations, which can offer clues to the cause of the disease and potential approaches to treatment. In a study published in Nature Communications, we have now uncovered several key events that occur in a common form of familial FTLD linked to mutations in the PGRN gene.

FTLD is a neurodegenerative disease caused in part by the build-up of protein aggregates in neurons. Different forms of FTLD—including the two major hereditary forms of the disease, FTLD-tau and FTLD-TDP—are associated with the build-up of different proteins. The hallmark of FTLD-tau is accumulated tau protein, a well-known player in neurological disorders such as Alzheimer's disease. The hallmark of FTLD-TDP (the form linked to defective PGRN) is the accumulation of TDP43, whose role in the disease is poorly understood.

One of the reasons less is known about FTLD-TDP is that the current disease models in mice look don't precisely mimic the way PGRN is mutated in patients. We developed a new model by introducing a mutation in PGRN gene associated with FTLD-TDP. In contrast to the earlier models, the mice in our study exhibit behavioral and cognitive symptoms that very closely mirror the disease pathology seen in humans.

Using this model, the researchers set out to understand what differentiates PGRN-mutant mice from their healthy counterparts. We focused their search on phosphorylation, a process that is normally involved in cell signaling but is often implicated in neurodegenerative diseases. Phosphorylated proteins have an additional chemical charge that can alter their behavior. In patients with FTLD-tau, as well as in Alzheimer's disease, phosphorylation of tau is thought drive its accumulation in neurons.

We performed a comprehensive search for proteins that are phosphorylated when PGRN is mutated. Notably, we discovered that tau is phosphorylated and specifically localized to synapse in the mutant mice. This was a surprising find, given that tau does not accumulate and have not been implicated in the TDP form of the disease (FTLD-TDP). Nevertheless, we found that phosphorylated tau causes synapse loss, and may disrupt communication between neurons. The toxic tau was seen as early as 4 weeks in mice, while TDP43 did not appear until 24 weeks—suggesting that tau may play a more important role early in the disease.

Highlight

Our study shows that tau phosphorylation (but not tau aggregation) is central to the pathology of both forms of FTLD as well as of Alzheimer's disease. In the case of FTLD-TDP, phosphorylated tau appears to drive early synapse changes long before TDP43 protein aggregates appear. The therapeutic implications of the study are clear, as the findings suggest that targeting tau phosphorylation may be an effective way to treat the disease at its earliest stages.

Over the course of the study, the team uncovered a number of molecular events that cause mutated PGRN to lead to tau phosphorylation. With many of the key signaling players now identified, the team is hopeful that future efforts can focus on possible treatment strategies for this form of the disease.

Tau pathway looks to be a very promising treatment target for familial FTLD associated with PGRN mutations. We're very excited to see how our findings will eventually translate into clinical improvement for these patients.

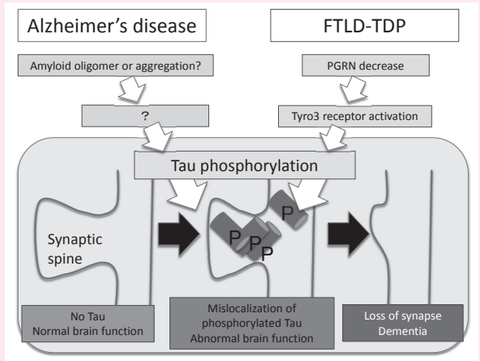


Fig. Tau phosphorylation is a common pathway across Alzheimer's disease, FTLD-tau and even FTLD-non-tau (FTLD-TDP).
Specific pathways of Alzheimer's disease and FTLD-non-tau (FTLD-TDP linked to PGRN mutations) merge at tau phosphorylation. The mislocalization of phosphorylated tau to dendritic spines degenerates postsynaptic spines and leads to loss of synapse function. The degeneration does not need the intracellular tau aggregation or extracellular amyloid aggregation (senile plaque). Hence the common pathology mediated by tau phosphorylation could initiate at the earliest stage of these degenerative diseases.

Publications

1. Fujita K, Chen X, Homma H, Tagawa K, Amano M, Saito A, Imoto S, Akatsu H, Hashizume Y, Kaibuchi K, Miyano S, Okazawa H. Targeting Tyro3 ameliorates a model of PGRN-mutant FTLD-TDP via tau-mediated synaptic pathology. ***Nat Commun***, 2018; 9: 433. doi: 10.1038/s41467-018-02821-z.
2. Kawahori K, Hashimoto K, Yuan X, Tsujimoto K, Hanzawa N, Hamaguchi M, Kase S, Fujita K, Tagawa K, Okazawa H, Nakajima Y, Shibusawa N, Yamada M, Ogawa Y. Mild maternal hypothyroxinemia during pregnancy induces persistent DNA hypermethylation in the hippocampal brain-derived neurotrophic factor gene in mouse offspring. ***Thyroid***. February 2018, ahead of print. https://doi.org/10.1089/thy.2017.0331
3. Fujita K, Mao Y, Uchida S, Chen X, Shiwaku H, Tamura T, Ito H, Watase K, Homma H, Tagawa K, Sudol M, Okazawa H. Developmental YAPdeltaC determines adult pathology in a model of spinocerebellar ataxia type 1. ***Nat Commun***, 2017; 8: 1864. doi: 10.1038/s41467-017-01790-z.
4. Okazawa H. Ultra-Early Phase pathologies of Alzheimer's disease and other neurodegenerative diseases. ***Proceedings of the Japan Academy***. Series B, Physical and biological sciences 93(6) 361-377 2017. doi: 10.2183/pjab.93.022.
5. Okazawa H. PQBP1, an intrinsically disordered/denatured protein at the crossroad of intellectual disability and neurodegenerative diseases. ***Neurochemistry international***, doi: 10.1016/j.neuint.2017.06.005.
6. Sato K, Kerever A, Kamagata K, Tsuruta K, Irie R, Tagawa K, Okazawa H, Arikawa-Hirasawa E, Nitta N, Aoki I, Aoki S. Understanding microstructure of the brain by comparison of neurite orientation dispersion and density imaging (NODDI) with transparent mouse brain. ***Acta radiologica open*** 6(4) 2058460117703816, doi: 10.1177/2058460117703816
7. Yamanishi, E., Hasegawa, K., Fujita, K., Ichinose, S., Yagishita, S., Murata, M., Tagawa, K., Akashi, T., Eishi, Y., Okazawa, H. A novel form of necrosis, TRIAD, occurs in human Huntington's disease. ***Acta Neuropathologica Communications***, doi: 10.1186/s40478-017-0420-1

Department of Pathological Cell Biology

Professor — Shigeomi SHIMIZU
Junior Associate Professor — Satoko ARAKAWA
Project Junior Associate Professor — Masatsune TSUJIOKA, Satoru TORII
Assistant professor — Shinya HONDA
Project Assistant Professor — Hirofumi YAMAGUCHI , Michiko MUROHASHI, Min Kyong SHIN, Nobuhiro FUJIKAKE, Hajime SAKURAI

This laboratory mainly focuses on understanding the molecular mechanisms of autophagy, programmed cell death, and the mitochondria-related diseases. We take biochemical, genetical, cellular biological approaches to elucidate them. The aims of this laboratory are to develop new strategies to control autophagy and cell death and to apply them to various diseases.

〈**Research Projects**〉

1, Analysis of Atg5/Atg7-independent alternative macroautophagy.

Atg5 and Atg7 are considered to be essential molecules for the induction of autophagy. However, we found that cells lacking Atg5 or Atg7 can still form autophagosomes/autolysosomes and perform autophagic protein degradation when subjected to certain types of stress. Unlike conventional autophagy, autophagosomes appeared to be generated in a Rab9-dependent manner by the fusion of the phagophores with vesicles derived from the *trans*-Golgi and late endosomes. Therefore, mammalian autophagy can occur via at least two different pathways; the Atg5/Atg7-dependent conventional pathway and an Atg5/Atg7-independent alternative pathway.

In this year, we analyzed the molecular mechanisms of genotoxic stress-induced alternative autophagy, and identified the essential role of p53 and damage-regulated autophagy modulator (Dram1). Dram1 was sufficient to induce alternative autophagy. In the mechanism of alternative autophagy, Dram1 functions in the closure of isolation membranes downstream of p53. These findings indicate that Dram1 plays a pivotal role in genotoxic stress-induced alternative autophagy. We also developed novel fluorescent probe DALgreen for monitoring autophagy in collaboration with Dojindo Laboratories. This probe exhibits fluorescence upon being incorporated into autophagosome and further enhances its fluorescence in acidic pH circumstance, which is favorable for monitoring autolysosome.

2, Molecular mechanisms of programmed cell death

Programmed cell death, which is required for the development and homeostasis of metazoans, includes mechanisms such as apoptosis, autophagic cell death, and necrotic death. Members of the Bcl2 family regulate apoptosis, among which Bax and Bak act as a mitochondrial gateway. Although embryonic fibroblasts from *bax/bak* double-knockout (DKO) mice are resistant to apoptosis, we previously demonstrated that these cells die through an autophagy-dependent mechanism in response to various types of cellular stressors. To determine the physiological role of autophagic cell death, we generated *atg5/bax/bak* triple-knockout (TKO) mice, in which autophagy is greatly suppressed compared with DKO mice. Embryonic fibroblasts and thymocytes from TKO mice underwent far less frequent autophagy, and their viability was much higher than DKO cells in the presence of certain cellular stressors, providing genetic evidence for the occurrence of Atg5-dependent death of DKO cells. Compared with wild-type embryos, loss of the interdigital web was significantly delayed in DKO embryos and further delayed in TKO embryos. Brain malformation is a remarkable feature of DKO embryos with a strain 129 genetic background, in contrast to mice with a B6 background, whereas such malformations appeared in TKO embryos even with a B6 background. Taken together, the data suggest that Atg5-dependent cell death contributes to embryonic development of DKO mice, implying that autophagy compensates for deficient apoptosis.

3, Analysis of mitochondrial diseases

Mitochondria play an essential role in both cell survival and cell death. In both cell function, mitochondrial membrane permeability is crucial. Therefore, we are focusing on channel proteins located on mitochondrial membrane. We are also focusing on mitochondrial diseases.

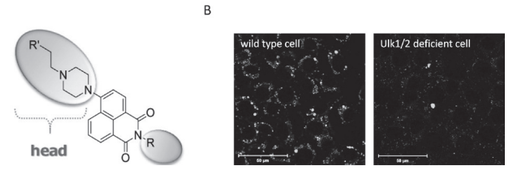


Figure1. Fluorescent small molecules for monitoring autophagic flux
(A) Structure of DALgreen. (B) DALgreen fluorescence was observed in chloroquine-treated wild-type cells, but not in Ulk1/Ulk2 double knockout cells, which is defective for the homologous functions of yeast Atg1 protein.

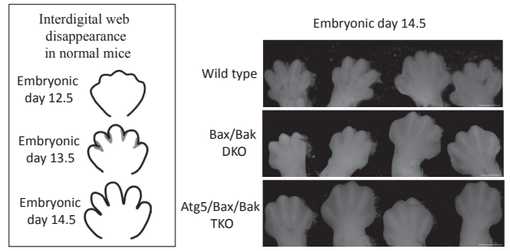


Figure2. Delay of interdigital web disappearance in *Atg5/Bax/Bak* TKO embryos and *Bax/Bak* DKO embryos
Schema of time-dependent interdigital web disappearance (left) and gross appearance of the interdigital webs of the limbs of indicated embryos on E14.5 (right).

List of Publications

[Original paper]

1. Role of Atg5-dependent cell death in the embryonic development of *Bax/Bak* double-knockout mice. *S. Arakawa, M. Tsujioka, T. Yoshida, H. Tajima-Sakurai, Y. Nishida, Y. Matsuoka, I. Yoshino, Y. Tsujimoto, S. Shimizu*. Cell death and differ 24: 1598-1608, 2017
2. Intrinsic autophagy is required for the maintenance of intestinal stem cells and for irradiation-induced intestinal regeneration. *J. Asano, T. Sato, S. Ichinose, M. Kajita, N.Onai, S. Shimizu, T. Ohteki*. Cell Reports 20: 1050-1060, 2017
3. Live Cell Imaging of Mitochondrial Autophagy with a Novel Fluorescent Small Molecule. *H. Iwashita, S. Torii, N. Nagahora, M. Ishiyama, K. Shioji, K. Sasamoto, S. Shimizu, K. Okuma*. ACS Chemical Biology 12: 2546-2551, 2017
4. Rhodium-catalyzed odorless synthesis of diaryl sulfides from borylarenes and S-aryl thiosulfonates. *K. Kanemoto, Y. Sugimura, S. Shimizu, S. Yoshida, T. Hosoya*. Chem. Commun. 53 : 10640-10643, 2017
5. Hyperoxidation of ether-linked phospholipids accelerates neutrophil extracellular trap formation. *S. Yotsumoto, Y. Muroi, T. Chiba, R. Ohmura, M. Yoneyama, M. Magarisawa, K. Dodo, N. Terayama, M. Sodeoka, R. Aoyagi, M. Arita, S. Arakawa, S. Shimizu, M. Tanaka*. Scientific Reports 7: Article number: 16026, 2017
6. The CCR4-NOT deadenylase complex controls Atg7-dependent cell death and heart function. *T. Yamaguchi, T. Suzuki, T. Sato, A. Takahashi, H. Watanabe, A. Kadowaki, M. Natsui, H. Inagaki, S. Arakawa, S. Nakaoka, Y. Koizumi, S. Seki, S. Adachi, A. Fukao, T. Fujiwara, T. Natsume, A. Kimura, M. Komatsu, S. Shimizu, H. Ito, Y. Suzuki, J.M. Penninger, T. Yamamoto, Y. Ima, K. Kuba*. Scientific Signalling 11: pii: eaan3638, 2018
7. Fluorescent small molecules for monitoring autophagic flux. *H. Iwashita, H. Tajima-Sakurai, N. Nagahora, M. Ishiyama, K. Shioji, K. Sasamoto, K. Okuma, S. Shimizu, Y. Ueno*. FEBS lett. *in press*, 2018

Department of Developmental and Regenerative Biology

Professor	Hiroshi Nishina, Ph.D.
Associate Professor	Jun Hirayama, Ph. D.
Assistant Professor	Norio Miyamura, Ph.D.
Research Assistant Professor	Erika Ishihara, Ph.D., Ruoxing Yu, Ph.D.

Our goal is to define the molecular basis for the mechanism of organ formation, regeneration, and maintenance using knockout mice and mutant fishes. To accomplish this goal, we have focused on defining signaling molecules and pathways that regulate liver and brain formation and maintenance. Our study will provide new insights into understanding the precise molecular mechanisms that underlie organ failures found in human disease and will lead to the development of new rational therapy for the diseases.

1. SAPK/JNK Signaling Pathway

Stress-activated protein kinase (SAPK)/c-Jun NH_2-terminal kinase (JNK) is activated by many types of cellular stresses and extracellular signals. We showed that two JNK activators, MKK4/SEK1 and MKK7, are required for full JNK activation and fetal liver formation. MKK4-deficient embryos die between E10.5 and E12.5 with impaired liver formation and massive apoptosis. MKK7-deficient embryos die between E11.5 and E12.5 with similar defects in liver formation. These results indicate that MKK4 and MKK7 cannot substitute for one another *in vivo* and that both are important for hepatoblast proliferation and survival during mouse embryogenesis. Furthermore, we found that the Tyr and Thr residues of JNK are sequentially phosphorylated by MKK4 and MKK7, respectively. We propose the hypothesis that the strong or weak activation of SAPK/JNK is induced by MKK4 and MKK7 to regulate the cell fate.

2. Hippo Signaling Pathway

The Yes-associated protein (YAP) is a transcriptional co-activator that contributes to the regulation of multiple cellular processes by activating several transcription factors. Recently, YAP was shown to play an important role in organ size control and to be inhibited by the Hippo signaling pathway. Either transgenic overexpression of YAP or knockout of Hippo pathway genes in mouse liver results in enlargement of this organ and the eventual development of hepatic tumors. *In vitro*, YAP overexpression promotes cell proliferation and induces oncogenic transformation by activating TEAD family transcription factors. YAP also plays a critical role in maintaining stem cell pluripotency, as knockdown of YAP leads to loss of this property in ES cells, and YAP overexpression suppresses ES cell differentiation. In certain tumor-derived cell lines exposed to various genotoxic stimuli, YAP promotes apoptosis via the induction of pro-apoptotic genes controlled by the transcription factor p73.

The functions of YAP are regulated by multiple post-translational modifications. Cell-cell contact triggers YAP inactivation via phosphorylation mediated by the Hippo pathway. The Hippo pathway component Lats kinase phosphorylates serine 127 (S127) of human YAP (hYAP), promoting its recognition and cytoplasmic retention by 14-3-3 protein. In addition, phosphorylation of hYAP S381 by Lats primes subsequent phosphorylation of hYAP S384 by casein kinase 1, leading to hYAP ubiquitination and degradation. We are studying on the regulation and function of YAP.

3. Circadian Clock

Circadian clocks are intrinsic, time-tracking systems that endow organisms with a survival advantage. The molecular mechanism underlying the circadian clock is based on interconnected transcriptional–translational feedback loops in which specific clock-factors repress transcription of their own genes. In vertebrate CLOCK and BMAL1 heterodimerize to form an active transcription complex that binds to promoter E-box elements, which are present in *Per* and *Cry* genes. Once PER and CRY proteins have been translated, they form a complex that inhibits CLOCK–BMAL1-mediated transcription. It is important to note that the CLOCK–BMAL1 heterodimer also stimulates the transcription of many other clock-controlled genes, which, in turn, influence functions external to the oscillatory mechanism itself and mediate the output of the clock. This accounts for the circadian rhythmicity of many physiological processes, such as food intake, hormonal synthesis and release, body temperature maintenance and several aspects of metabolism. The principal cue that influences circadian rhythmicity is light. The molecular pathways that light uses to influence the clock mechanism are thereby of central importance. We have been studying the light signaling pathway for circadian transcription and clock entrainment using zebrafish as the model animal.

Highlight

The presence of senescent, transformed or damaged cells can impair tissue function or lead to tumorigenesis; therefore, organisms have evolved quality control mechanisms to eliminate them. Here, we show that YAP activation induced by inactivation of the Hippo pathway specifically in damaged hepatocytes promotes their selective elimination by using *in vivo* mosaic analysis in mouse liver. These damaged hepatocytes migrate into the hepatic sinusoids, undergo apoptosis and are engulfed by Kupffer cells. In contrast, YAP activation in undamaged hepatocytes leads to proliferation. Cellular stresses such as ethanol that damage both liver sinusoidal endothelial cells and hepatocytes switch cell fate from proliferation to migration/apoptosis in the presence of activated YAP. This involves the activation of CDC42 and Rac that regulate cell migration. Thus, we suggest that YAP acts as a stress sensor that induces elimination of injured cells to maintain tissue and organ homeostasis.

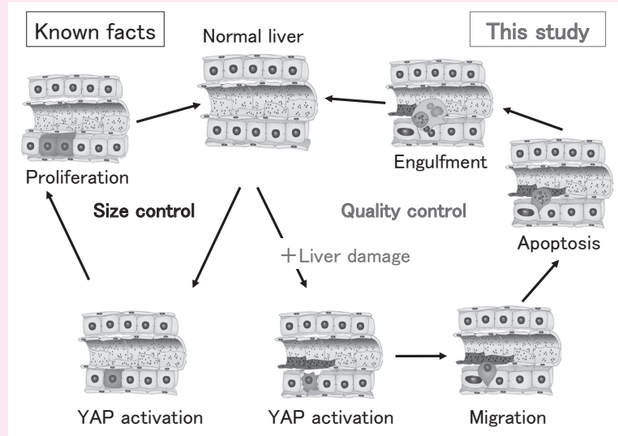


Fig.1.Schematic Model of the Change in Mouse Hepatocyte Fate

Publications

1. Norio Miyamura, Shoji Hata, Tohru Itoh, Minoru Tanaka, Miki Nishio, Michiko Itoh, Yoshihiro Ogawa, Shuji Terai, Isao Sakaida, Akira Suzuki, Atsushi Miyajima and Hiroshi Nishina (2017) YAP determines the cell fate of injured mouse hepatocytes *in vivo*. ***Nature Communications*** 8, 16017.
2. Tokiwa Yamasaki, Norie Deki-Arima, Asahito Kaneko, Norio Miyamura, Mamiko Iwatsuki, Masato Matsuoka, Noriko Fujimori-Tonou, Yoshimi Okamoto-Uchida, Jun Hirayama, Jamey D. Marth, Yuji Yamanashi, Hiroshi Kawasaki, Koji Yamanaka, Josef M. Penninger, Shigenobu Shibata and Hiroshi Nishina. (2017) Age-dependent motor dysfunction due to neuron-specific disruption of stress-activated protein kinase MKK7. ***Scientific Reports*** 7, 7348.
3. Tatsuyuki Matsudaira, Kojiro Mukai, Taishin Noguchi, Junya Hasegawa, Tomohisa Hatta, Shunichiro Iemura, Tohru Natsume, Norio Miyamura, Hiroshi Nishina, Jun Nakayama, Kentaro Semba, Takuya Tomita, Shigeo Murata, Hiroyuki Arai and Tomohiko Taguchi (2017) Endosomal phosphatdyl-serine is critical for the YAP signalling pathway in proliferating cells. ***Nature Communications*** 8, 1246.
4. Kohei Otsubo, Hiroki Goto, Miki Nishio, Koichi Kawamura, Shigehisa Yanagi, Wataru Nishie, Takehiko Sasaki, T, Tomohiko Maehama, Hiroshi Nishina, Koshi Mimori, Toru Nakano, Hiroshi Shimizu, Tak W. Mak, Kazuwa Nakao, Yoichi Nakanishi, Akira Suzuki (2017) MOB1-YAP1/TAZ-NKX2.1 axis controls bronchioalveolar cell differentiation, adhesion, and tumor formation. ***Oncogene*** 36, 4201-4211.
5. Seiichiro Mori, Takamasa Takeuchi, Yoshiyuki Ishii, Takashi Yugaw, Tohru Kiyono, Hiroshi Nishina, Iwao Kukimoto. (2017) Human Papillomavirus 16 E6 Up-regulates APOBEC3B via the TEAD Transcription Factor. ***J. Virology*** e02413-16
6. Jun Negishi, Yuka Omori, Mami Shindo, Hayate Takanashi, Shiori Musha, Suminori Nagayama, Jun Hirayama, Hiroshi Nishina, Takashi Nakakura, Chihiro Mogi, Koichi Sato, Fumikazu Okajima, Yuta, Mochimaru, and Hideaki Tomura (2017) Manganese and cobalt activate zebrafish ovarian cancer G-protein-coupled receptor 1 but not GPR4. ***Journal of Receptors and Signal Transduction*** 37, 401-408.
7. Junichi Maruyama, Kazutoshi Inami, Fumiyoshi Michishita, Xinliang Jiang, Hiroaki Iwasa, Kentaro Nakagawa, Mari Ishigami-Yuasa, Hiroyuki Kagechika, Norio Miyamura, Jun Hirayama, Hiroshi Nishina, Daichi Nogawa, Kouhei Yamamoto and Yutaka Hata (2017) Novel YAP1 Activator, Identified by Transcription-Based Functional Screen, Limits Multiple Myeloma Growth. ***Molecular Cancer Research*** 10.1158/1541-7786.
8. Yoichi Asaoka, Hiroshi Nishina, Makoto Furutani-Seiki (2017) [review] YAP is essential for 3D organogenesis withstanding gravity. ***Develop. Growth Differ*** 59: 52-58.

Department of Stem Cell Biology

Professor	Emi K. Nishimura, M.D., Ph. D.
Associate Professor	Daisuke Nanba, Ph D.
Assistant Professor	Hiroyuki Matsumura, Ph. D.
Project Assistant Professor	Yasuaki Mohri, Ph. D., Hironobu Morinaga, Ph. D., Kyosuke Asakawa, Ph. D.

Stem cell systems play fundamental roles in tissue turnover and homeostasis. Our goal is to understand the mechanisms of tissue homeostasis driven by stem cell systems and to apply the knowledge to better understand the mechanisms underlying specific tissue decline by aging, cancer development and other diseases associated with aging. We further aim to apply this knowledge to drug discovery and regenerative medicine using somatic stem cells and the prevention and treatment of age-associated diseases.

1) Identification of stem cells in the skin

The skin is the largest organ in the body. Hair follicles in the skin constantly renew themselves by alternate phases of growth, regression and rest. During this process, mature melanocytes (pigment cells) in hair follicles are replaced by a new cell population every hair cycle. We previously identified the source of those melanocytes, "melanocyte stem cells" (McSC), which are located in the hair follicle bulge and supply mature melanocytes required for hair and skin pigmentation (Nishimura EK et al. Nature 2002). We currently identified McSCs in eccrine sweat glands in non-hair-bearing skin areas as well as a potential melanoma-initiating cells (Okamoto N et al. PCMR, 2014). Also we are currently searching for the prospective method for identification of epidermal keratinocyte stem cells in mouse and human skin.

2) Mechanisms of stem cell maintenance

The underlying mechanisms of stem cell maintenance is a fundamental issue in stem cell biology and medicine. We previously demonstrated that the niche microenvironment plays dominant role in melanocyte stem cell fate determination (Nishimura EK et al. 2002). We then revealed that hair follicle stem cells (HFSC), which surround McSCs in the hair follicle bulge-subbulge area, serve as a functional niche for McSC maintenance through transforming growth factor β (TGF-β) (Nishimura EK et al. Cell Stem Cell, 2010) (Tanimura S et al. Cell Stem Cell 2011). As intrinsic defects in stem cells such as caused by *Mitf* or *Bcl2* deficiency also induces McSC depletion which leads to the progressive expression of hair graying phenotype, incomplete maintenance of McSCs either by defective signaling from the stem cell niche or by intrinsic defects in stem cells induces the progressive hair graying phenotype.

3) Mechanisms for stem cell aging and tissue/organ aging

Physiological hair graying is the most obvious outward sign of aging in mammals, yet it has been unclear what causes the incomplete maintenance of MsSCs during the course of aging (Nishimura EK et al. Science 2005). We have found that genotoxic stress abrogates renewal of McSCs by triggering their differentiation without inducing stem cell apoptosis nor cellular senescence. Our findings indicated that a "stem cell renewal checkpoint" exists to maintain the quality of the melanocyte stem cell pool (Inomata K, Aoto T et al. Cell 2009). Similar checkpoint has been found in other somatic stem cell systems as well. We recently found that HFSCs also have similar checkpoint mechanism. We are currently studying the underlying molecular mechanism.

4) Hair follicle aging is driven by stem cell-centric aging program

Hair thinning/loss is a prominent aging phenotype but has an unknown mechanism. We have shown that hair follicle stem cell (HFSC) aging causes the stepwise miniaturization of hair follicles and eventual hair loss in wild-type mice and in humans. *In vivo* fate analysis of HFSCs revealed that the DNA damage response in HFSCs causes proteolysis of Type XVII Collagen (COL17A1/BP180), a critical molecule for HFSC maintenance, to trigger HFSC aging, characterized by the loss of stemness signatures and by epidermal commitment. Aged HFSCs are cyclically eliminated from the skin through terminal epidermal differentiation, thereby causing hair follicle miniaturization. The aging process can be recapitulated by *Col17a1*-deficiency and prevented by the forced maintenance of COL17A1 in HFSCs, demonstrating that COL17A1 in HFSCs orchestrates the stem cell-centric aging program of the epithelial mini-organ (Matsumura H et al. Science 2016).

5) Development of skin regeneration technology with human skin stem cells and/or stem cell-targeted small molecules

Human epidermal keratinocyte stem cells can be cultivated under suitable conditions, and generate a progeny large enough to entirely reconstitute the epidermis of an adult human. This has enabled the autologous transplantation of cultured epidermal sheets onto patients with extensive burns. However, the cultured keratinocytes can regenerate only the epidermis and cannot suppress dermal scarring. To develop novel skin regeneration technology, we have investigated human epidermal keratinocytes and found that human epidermal keratinocyte stem cells can be identified *in situ* by analyzing cell motion during their cultivation (Nanba et al., J. Cell Biol., 2015). The identification of keratinocyte stem cells by image analysis is a valid parameter for quality control of cultured keratinocytes for transplantation will be able to improve the clinical outcome of cell therapy and the efficiency of cell manufacturing for regenerative medicine. Finally, the treatment of skin ulcer and decubitus is an urgent problem in this aging society. We are currently trying to establish the screening system for small molecules that activate stem cells in the wound edge.

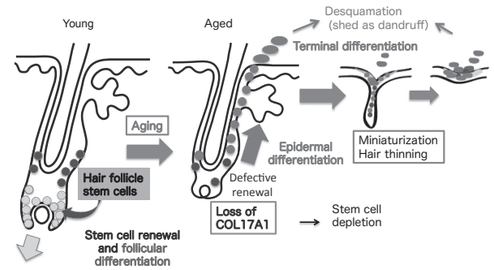


Figure. The mechanisms of hair follicle aging and hair thinning.

Invited lecture/presentation at international meetings

Emi K.Nishimura : Stem cell-centric Mechanisms of hair follicle aging : Gordon Research Conference-Cornea and Ocular Surface Biology and Pathology- : (California, USA) Feburuary 18-23, 2018

Emi K. Nishimura : Stem cells orchestrate hair follicle aging program : Internatioal Meeting on RECQ Helicases and Related Disease 2018 : (Kazusa Akademia Hall: Chiba) February 16-18, 2018

Emi K.Nishimura : Stem cell orchestrate hair follicle aging : JSPS&NUS Joint 2nd Symposium "New Horizons in Normal And Cancer Stem Cell Research" : (Kumamoto：熊本大学山崎記念館) : January 18-20, 2018

Emi K. Nishimura : Stem cells orchestrate hair follicle aging program:Stem Cells in Disease Modeling and Therapeutics:(Tokyo : Yayoi Auditorium, University of Tokyo) November 13, 2017

Emi K. Nishimura : The mechanism of aging-associated hair graying and hari thinning:toward the discovery of pharmacological targets:(Kyoto:Kyoto International Conference Cente):Octobar 31 - November 3, 2017

Emi K. Nishimura : Melanocyte stem cell in eccrine sweat glands: a potential original of acral melanoma:IPCC 2017(The International Pigment Cell Conference): (Denver, USA) August 26-30th, 2017

Emi K. Nishimura : Stem cells orchestrates hair follicle aging program : ISSCR(International Society for Stem Cell Research 2017 Annual Meeting) : (Boston, USA)June 14-17th, 2017

Emi K. Nishimura : Tissue aging program based on stem cell aging in hair follicle : KEYSTONE SYMPOSIA-Aging and Mechanisms of Aging-Related Disease- (神奈川：パシフィコ横浜) May 15-19, 2017

Department of Immunology

Professor	Takeshi Tsubata, M.D., Ph.D.
Associate Professor	Takahiro Adachi, Ph.D.
Lecturer	Ji-Yang Wang, Ph.D.
Assistant Professors	Naoko Matsubara, Ph.D. Chizuru Akatsu, Ph.D.
Researchers	Mohammad Aslam, Ph.D.
	Medhzidov Nazim, Ph.D.

Normal immune system removes pathogens and cancer cells but does not respond to non-microbial foreign substances or normal self-antigens. Immune responses to non-microbial foreign substances and self-antigens cause allergy and autoimmune diseases, respectively. Immune responses to non-protein antigens play crucial roles in host defense against pathogens such as tuberculosis bacilli and meningococci, and autoimmune diseases such as lupus and immuno-neurological disorders. The mechanisms for immune responses to non-protein antigens are distinct from those to protein antigens, but are largely unknown. Thus, immune responses to non-protein antigens constitute a remaining frontier in immunology research. Followings are our research subjects.

1) Elucidation of the mechanisms for humoral immune responses to glycans, glyco-lipids and nucleic acids-related antigens.
2) Elucidation of the mechanisms for the regulation of autoantibody production to glycolipids and nucleic acid-related antigens involved in development of Guillain-Barre syndrome and systemic lupus erythematosus, respectively.
3) Elucidation of the role of glycan signals in the regulation of humoral immune responses, and development of modified glycan signals for therapy.
4) Elucidation of the role of reactive oxygen species (ROS) and membrane trafficking in B lymphocyte activation
5) Development of therapeutic vaccines that replaces therapeutic antibodies.

1. Development of the method for identification of CD22 ligands, and identification of novel CD22 ligands

CD22 (also known as Siglec2) is a membrane molecule mostly expressed in B lymphocytes (B cells). CD22 recognizes sialic acid at the extracellular lectin domain, and activates SH2-containing protein tyrosine phosphatase 1 (SHP-1) at the cytoplasmic region, thereby negatively regulating signaling through B cell antigen receptor (BCR). Various membrane-bound lectins including CD22 interact with ligand-containing glycoproteins and glycolipids expressed on the same cell (cis-ligands). CD22 is functionally regulated by cis-ligands. However, how cis-ligands regulate CD22 is poorly understood. Due to weak interaction of lectins to glycan ligands, isolation of glycan ligands by conventional methods such as immunoprecipitation is not usually possible. We demonstrate that CD22 ligands are efficiently identified by proximity labeling using tyramide (see Highlight), and indeed isolated a novel CD22 ligand.

2. Development of sialic acid derivatives carrying an adjuvant activity.

Various immuno-stimulatory compounds are used as adjuvants. These compounds augment antibody production by stimulating innate immune cells thereby augmenting activation of T lymphocytes. However, activation of innate immune cells causes adverse effect by inducing inflammation. By collaborating with Profs. Kiso and Ishida at Gifu University, we developed synthetic sialosides that binds to CD22 with high affinity. These compounds reverse CD22-mediated suppression of B cell responses to various stimuli such as T cell derived CD40L and stimuli from microbes such as lipopolysaccharides, leading to augmented antibody production to immunization. In contrast, these compounds do not induce inflammation because they augment activation of B lymphocytes but not innate immune cells. Thus, these compounds will be useful as a safe adjuvant with no ability to induce inflammation.

3. Studies on the role of reactive oxygen species (ROS) in B lymphocyte activation

Reactive oxygen species (ROS) are not only toxic substances inducing oxidative stress, but also play a role as a second messenger in signal transduction through various receptors. We demonstrate that BCR ligation induces biphasic ROS production: Transient ROS production is followed by sustained and robust ROS production at 2-6 hours after BCR ligation. We further demonstrate that ROS production in the late but not early phase is essential for B cell proliferation.

Highlight

Development of a method to identify CD22 cis-ligands and elucidation of stepwise recruitment of cis-ligands to CD22.

Many membrane-bound lectins associate with the glycan ligands expressed on the same cell (cis-ligands). CD22 is known to be regulated by cis-ligands. Because bindings of lectins to glycan ligands are weak, identification of glycan ligands by conventional methods such as immunoprecipitation is often difficult. We demonstrated that CD22 cis-ligands are easily identified by proximity labeling using tyramide.

By using proximity labeling, we elucidated stepwise association of CD22 with its cis-ligands. CD22 cis-ligands are located in relative proximity of CD22 by a mechanism independent of glycan-lectin interaction. CD22 then recruit cis-ligands to more proximity by its glycan-binding activity (Figure 1).

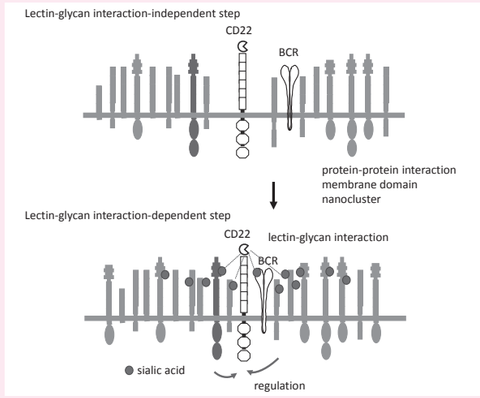


Figure1. Stepwise recruitment of cis-ligands to CD22
CD22 cis-ligands are located in relative proximity of CD22 by a mechanism independent of glycan-lectin interaction (upper panel). CD22 then recruit cis-ligands to more proximity by its glycan-binding activity (lower panel).

Publications

[original papers]

1. Liu, J., Zhu, H., Qian, J., Xiong, E., Zhang, L., Chu, Y., Kubagawa, H., Tsubata, T. and Wang, J.-Y. (2018): FcμR promotes the survival and activation of marginal zone B cells and protects mice against bacterial sepsis. *Front. Immunol.* 9: 160.
2. Alborzian Deh Sheikh, A., Akatsu, C., Imamura, A., Abdu-Allah, H. H. M., Takematsu, H., Ando, H., Ishida, H. and Tsubata, T. (2018): Proximity labeling of cis-ligands of CD22/Siglec-2 reveals stepwise α2,6 sialic acid-dependent and -independent interactions. *Biochem. Biophys. Res. Comm.* 495: 854-859.
3. Tsubata, T. (2018): Negative regulation of B cell responses and self-tolerance to RNA-related lupus self-antigen. *Proc. Jpn. Acad. Ser. B Phys.* Biol. Sci. 94 (1) :35-44 .
4. Liu, J., Xiong, E., Zhu, H., Mori, H., Yasuda, S., Kinoshita, K., Tsubata, T. and Wang, J.-Y. (2017): Efficient induction of Ig gene hypermutation in ex vivo-activated primary B cells. *J. Immunol.* 199(9):3023-3030.
5. Tsubata, T (2017): B cell tolerance and autoimmunity. *F1000Research* 6 (F1000 Faculty Rev.): 391.

Department of Molecular Pathogenesis

Professor	Akinori Kimura, M.D., Ph.D.
Associate Professor	Takeharu Hayashi, M.D., Ph.D.
Assistant Professor	Jianbo An, PhD
Research Associate	Taeko Naruse, Ph.D.

Genetic factors, i.e. functional diversity of human genome mainly due to mutations or polymorphisms, are involved in the etiology and pathogenesis of human diseases. Contribution of gene mutations in the disease development is quite large in monogenic diseases showing simple Mendelian inheritance, while multiple gene mutations along with environmental factors are involved in the pathogenesis of multi-factorial diseases. Our goal is to decipher the disease-related genes for intractable or incurable diseases for which etiology and/or pathogenesis are not fully understood and, in addition, to develop novel therapeutic or preventive strategies for the intractable diseases based on the knowledge of disease-related genes.

1. Molecular mechanisms for hereditary cardiomyopathy

We have generated transgenic lines over-expressing M21 (M21-Tg). M21 is an inhibitory molecule of myosin light chain phosphatase and we found that calcium sensitivity of cardiac muscle contraction was increased in M21-Tg and M21-Tg showed cardiac phenotypes, cardiac hypertrophy, myocardial disarrays and later development of cardiac failure, similar as those found in patients with hypertrophic cardiomyopathy (HCM). M21-Tg will be a useful model for development of therapeutics for HCM.

2. Molecular mechanisms commonly found for atherosclerosis and inflammatory bowel disease

We generated a transgenic mouse line expressing coronary atherosclerosis-associated MKL1 under the CD68 promoter (MKL1-Tg) and found that these mice exhibited abnormality in function of macrophages. In addition, MKL1-Tg spontaneously develop symptoms similar to inflammatory bowel disease. Furthermore, we found that MKL1-Tg was susceptible to DSS-induced experimental colitis, suggesting a common pathological contribution of abnormal development of macrophages in atherosclerosis and inflammatory bowel disease.

3. Molecular mechanisms for Takayasu aortitis

In a collaboration study, we searched for susceptibility genes for Takayasu aortitis.

4. Analysis of MHC genes in human and animals

We have analyzed HLA genes and identified specific DRB1 alleles controlling anti MDA-5 antibodies in patients with dermatomyositis. In addition, we continue to investigated MHC class I diversities in macaque model for SIV vaccination in detail.

5. Genome diversity in association with HIV/AIDS

We have investigated natural selection on immune-related genes in the primate evolution. This year, we revealed that OAS1 polymorphisms were associated with viral load after HIV-1 infection.

Highlight

We have generated a transgenic mice overexpressing human MKL1 gene under CD68 promoter (MKL1-Tg), because we previously demonstrated higher expression of MKL1 in activated macrophage was associated with atherosclerosis. In addition, loss of MKL1 expression was recently reported to prevent DSS-induced colitis in mice, a well-known experimental model of inflammatory bowel disease (IBD). It also is known that IBD patients are at high risk of atherosclerosis. We then investigated MKL1-Tg for possible susceptibility to IBD. We first demonstrated that higher expression of MKL1 in colon in the DSS-induced colitis. MKL1-Tg spontaneously develop colitis phenotypes such as rectal prolapse, shortened colon with cryptitis. We also found that MKL1-Tg showed a skewed differentiation of macrophages toward M1 phenotypes. It also was demonstrated that MKL1-Tg was susceptible to DSS-induced colitis. These observations indicated that MKL1 crucially contribute to the development of colitis via regulating function of macrophages, suggesting a potential therapeutic target for preventing IBD.

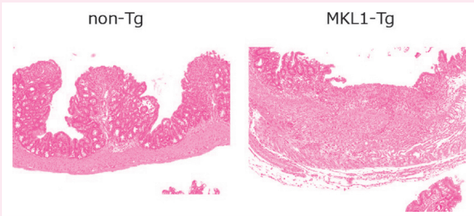


Figure Pathological findings in colons from MKL1-Tg and control mice
MKL1-Tg showed a stronger susceptibility to DSS treatment than control mice, demonstrating severer inflammation with infiltration of lymphocytes.

Publications

(#; equal contribution, *; corresponding author)

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Division of Medical Genomics

Over the last decade, remarkable strides have been made in human genome science. The complete human genome sequences have been available through the public databases, and many high throughput technologies have been developed in the human genome project. In the post-sequence era we have excellent opportunities to identify genetic or epigenetic changes that are responsible for diseases. The missions of the Division of Medical Genomics are to understand genomic, epigenomic and proteomic changes underline the initiation and progression of human disease, and to elucidate the pathogenesis of intractable diseases. Our goal is to translate our research discoveries into the clinical setting.

【Molecular Cytogenetics】

The principal aim of our research is to understand the molecular basis underlying cancer and genetic diseases including multiple congenital anomalies/intellectual Disabilities (MCA/ID). We have contributed as follows;

1. Identification of novel genes including microRNAs responsible for cancer and the development of miRNA-based therapeutics in cancer.
2. Understanding the pathogenesis of intractable cancers and genetic disorders based on the integrative omics.
3. Establishment of diagnostic devices for the implementation of precision medicine in cancer and genetic disorders.

【Molecular Genetics】

We aimed to analyze functions of BRCAs and other breast cancer-related molecules and reveal the mechanism of breast carcinogenesis.

1. We sought novel synthetic lethal interactions between BRCA2 and chemical compounds provided by Chemical Biology Screening Center at TMDU.
2. FK506 binding protein 51 (FKBP51), a member of the immunophilin family, is involved in tumorigenesis, and chemoresistance. We demonstrate that FKBP51 positively controls cell motility by promoting RhoA and ROCK activation.
3. We have focused on the exosome and found that the driver gene mutation of a primary tumor cell would cause organotropic adsorption of exosome into the tissue and construct pre-metastatic niche.

【Molecular Epidemiology】

1. We are studying the mechanism how metabolism of F1 is affected by intrauterine environment using DOHaD model mice.
2. We established mother and birth cohort, BC-GENIST, to study health related epigenome markers in collaboration with TMDU hospital.
3. We identify WIPF3 and LIPA genes as candidates for the abdominal aortic aneurism risk in an exome wide association study.

【Genomic Pathology】

1. We performed immuno-genomics analysis of tumor infiltrating lymphocytes in gastric cancer and identified that Sulfated glycosaminoglycans are major and functional B cell antigens in cancers. In addition, we successfully created antibodies with antitumor activity by immune-genomics analysis.
2. We developed an algorithm to identify lymph node metastasis of breast cancer from histopathological tissue images using deep learning. We won the 3rd place in the international competition (ISBI 2017 Camelyon 17 workshop) using this algorithm. We are investigating relationships between genetic abnormalities in cancer cells and their cellular and architectural abnormalities from digital pathological image and the corresponding genomic information using the developed algorithm.
3. We have developed an algorithm for the global profiling of cancer-stromal interactions by massively-parallel sequencing of cancer xenograft transcriptome. We are analyzing patient-derived xenograft (PDX) samples, where clinical cancer tissue is directly transplanted into immune-compromised mouse.

【Epigenetics】

1. We reported the existence of sushi-ichi-related retrotransposon homologue family of genes (*SIRH* family genes) and demonstrated that they play placental essential eutherian-specific functions, such as *Peg10, Peg11/Rtl1 and Sirh7*, or important brain functions, such as *Sirh11, Sirh3 and Sirh8*.
2. Epigenetic properties of cultured embryonic stem cells (ESCs), including DNA methylation imprinting, are important because they affect the developmental potential. We pointed out problems of ES cell culture media currently used and proposed an improved culture media,
3. By performing single embryos RNA-seq analysis on human blastocysts fertilized by intracytoplasmic sperm injection, we identified a number of genes in which the expression levels are lowered with increasing maternal age, such as important genes for meiotic chromosomal segregation and for embryo implantation.

【Medical Science Mathematics】

1. We participated in the international consortium for asthma (TAGC), a striking progress in big medical-omic analysis, and found five novel loci related to asthma through GWAS meta-analysis. These largely overlapped with loci for autoimmune and inflammatory diseases, which suggests roles in regulating the immune system.
2. Taking hereditary diabetes cases as an example, we established a method to identify the relationship between the mutations in specific protein domains and the severity of disease by enrichment analysis, and identify associations between the mutations and their protein structures. Furthermore, now we are extending our methodologies from whole-exome sequencing to whole-genome sequencing to explore novel disease-causing genes.

Department of Molecular Cytogenetics

Professor	Johji Inazawa, M.D., Ph.D.
Lecturer	Jun Inoue, Ph.D.
Assistant Professor	Yasuyuki Gen, M.D., Ph.D.
Assistant Professor	Tomoki Muramatsu, Ph.D.
Research Assistant Professor	Daniela Tiaki Uehara, Ph.D.

The principal aim of the Department of Molecular Cytogenetics is to understand the molecular mechanisms underlying cancer and genetic diseases including multiple congenital anomalies and/or intellectual disability (MCA/ID). Our research interests are as follows: (1) Identification of genes responsible for cancer and unknown genetic diseases, (2) Development of innovative techniques for the detection of genomic and epigenomic aberrations underlying the pathogenesis of cancer and genomic disorders, and (3) Establishment of practically useful modalities for diagnosis and therapy in personalized medicine in cancer and other intractable diseases. Our goal is to bridge the gap between basic and clinical research for the benefit of individual patients.

I. Precision cancer medicine based on omics and functional research

1. Molecular basis for the development of novel strategies to inhibit tumor metastasis

Cancer metastasis is a multistep process, involving genetic and epigenetic events that result in the activation of metastasis-associated genes, together with the activation of oncogenes and/or the inactivation of tumor suppressor genes. However, the underlying mechanisms of metastasis are still poorly understood. Recently, we have generated a subclone with highly-metastatic potential to lung from KYSE150 cells, an esophageal squamous cell carcinoma (ESCC) cell line, and found that the promotion of cytokine/chemokine secretion may contribute to lung metastasis of ESCC (Okuda et al. Oncotarget 2017).

2. Molecular basis for cell metabolism-based personalized cancer medicine

Cellular metabolism is rewived by aberrations of metabolism-related genes in human cancers. Recently, we have found that high expression of *miRNA-432-3p* leads to constitutive activation of NRF2, a transcriptional factor involved in cellular metabolism in ESCC (Akdemir et al. Mol Cancer Res. 2017). Furthermore, we have demonstrated that glutamine synthetase (GS) is down-regulated in ovarian cancers, and depletion therapy of extracellular glutamine may be a useful strategy against GS^{low}-ovarian cancer (Furusawa et al. Carcinogenesis 2018 doi: 10.1093/carcin/bgy033).

II. Cancer omics research

Though the "tailor-made Medical treatment Program", we have explored tumor susceptible genes and cancer biomarkers of malignancy for esophageal squamous cell cancer (with Tokyo Med. Dent. Univ. and Aichi Cancer Center), breast cancer (with Cancer Institute Hospital and the Univ. of Tokushima), pulmonary cancer (Nagoya Univ. and Shiga Univ. of Medical Science), colorectal cancer (with CIH and Osaka Univ.), prostate cancer (with Kyoto Univ. and Iwate Medical Univ.) and gastric cancer (with National Cancer Center and UT) in order to establish a personalized cancer medicine.

Furthermore, under the "Project for development of innovative research on cancer therapeutics (P-DIRECT)", we have performed an integrative analysis of genomics, epigenomics and gene expression in esophageal squamous cell cancers. Recently, we performed genome-wide screening of DNA methylation status in 67 primary esophageal squamous cell carcinoma tissues for identification of biomarkers as a predictor of lymph node metastasis. As a result, we found that the DNA methylation status of *HOXB2* and *SEPT9* were associated with the presence of lymph node metastasis (Nagata et al., Oncotarget 2017). Furthermore, we identified *miR-509-5p* and *miR-1243* as EMT suppressive microRNA (miRNA) using a cell-based reporter system combined with miRNA library composed of 1090 miRNAs (Hiramoto et al. Sci Rep 2017).

III. Molecular investigation of congenital disorders

Intellectual disability (ID) is a heterogeneous condition affecting 2-3% of the population, often associated with multiple congenital anomalies (MCA). The genetic cause remains largely unexplained for most cases. Since 2005, we have been investigating the causes of ID/MCA of unknown etiology in 645 subjects through the use of chromosomal microarrays. First, we performed a two-stage screening by two in-house bacterial artificial chromosome (BAC) arrays, which identified pathogenic copy number variants (CNVs) in 133 patients. Next, we performed a third screening by SNP arrays in 450 negative cases from the previous screenings, and smaller causative CNVs were detected in 22 subjects. Overall, our three-stage screening allowed the identification of pathogenic CNVs in 155 subjects, which means that 24% of the cases can be explained by alterations in the copy-number state (Uehara et al. J Hum Genet. 2016). Through this project, we also carried out a parallel research following the identification of the *CASK* gene as a cause of ID and microcephaly with pontine and cerebellar hypoplasia (MICPCH). We recruited 41 additional MICPCH patients and identified causative or candidate genomic aberrations in 37, thus clarifying the etiology in 90% of the cases (Hayashi et al. PloS ONE 2017).

Articles

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Department of Molecular Genetics

Professor	Yoshio Miki, MD. Ph.D.
Associate Professor	Akira Nakanishi, Ph.D.
Assistant Professor	Miho Takaoka, Ph.D.
Project Assistant Professor	Shigeaki Sunada Ph.D.

BRCA 1 and 2, which are responsible genes for hereditary breast and ovarian cancers, function for DNA double strand break repair. While they are guardians to prevent carcinogenesis, they repair DNA damage by anticancer drugs in cancer tissues, thereby suppressing cell death and providing resistance to treatment. Moreover, they have many other functions to maintain DNA stability, and these disorders also lead to the onset of breast cancer. Therefore, we will pursue the functions of breast cancer-related molecules such as BRCA 1 · 2 and clarify the mechanism of mammary carcinogenesis, and work on the development of novel therapeutics for breast cancer.

1. FKBP51 regulates cell motility and invasion via RhoA signaling

We discovered two novel interacting partner proteins of FKBP51 using immunoprecipitation and mass spectrometry: deleted in liver cancer 1 (DLC1) and deleted in liver cancer 2 (DLC2). DLC1/2 are Rho GTPase-activating proteins that are frequently down-regulated in various cancers. We demonstrated that overexpression of FKBP51 enhances cell motility and invasion of U2OS cells via up-regulation of RhoA activity. Moreover, FKBP51-depleted cells displayed a cortical distribution of actin filaments and decreased cell motility and invasion. Considered together, our results demonstrate that FKBP51 positively controls cell motility by promoting RhoA and ROCK activation; thus, we have revealed a novel role for FKBP51 in cytoskeletal rearrangement and cell migration and invasion.

2. Mechanism of organotropic cancer metastasis via gene mutation

Our clinical investigation has found several driver mutations from genomic analysis between the primary and metastatic tumors of breast cancer patients. These achievements are certainly similar to the previous results. On the other hand, we have noticed these driver mutations tend to cause organotropic metastasis. Then, we have focused on the exosome, which is one of the extracellular vesicles and plays a role of intercellular communication, as a prospective promotion factor for the organotropic metastasis. First, we silenced MGA (lab name), one of metastatic driver genes, in MCF7 (human breast cancer cell line) and collected exosome from culture supernatant. We found that some integrin subunits in exosome were upregulated and these had high binding affinity to the cells which formed an organ. Next, MGA defective cancer cell-derived exosome were added into normal cells and gene expression analysis were conducted by microarray. Then, we also found that the exosome activated some tumor malignancy-related genes. These findings indicate that the driver gene mutation would cause organotropic adsorption of exosome into the tissue and construct pre-metastatic niche at the site as an environment where the metastasis of a primary tumor promotes (Fig.2). Further investigation of the mechanism will be needed using animal model and our study would develop a method of metastasis prevention for clinical applications.

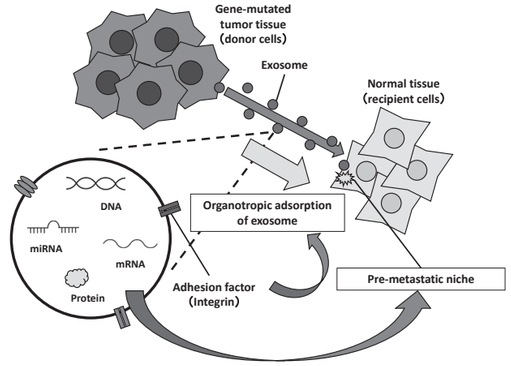

Figure 2. Gene mutation-derived organotropic cancer metastasis

Highlight

FKBP51 regulates RhoA-ROCK signaling

FK506 binding protein 51 (FKBP51), a member of the immunophilin family, is involved in multiple signaling pathways, tumorigenesis, and chemoresistance. FKBP51 expression correlates with metastatic potential in melanoma and prostate cancer. However, the functions of FKBP51, particularly involving the regulation of cell motility and invasion, are not fully understood.

To focus on the mechanism by which FKBP51 promotes in vitro cell motility and invasion of cancer cells, we investigated the potential downstream targets of Rho activity. There are two major effectors for Rho signaling, ROCK and mDia. The balance of these two signaling molecules determines stress fiber formation and membrane ruffles. Rho-mDia signaling produces membrane ruffles through Rac activation, and this signaling is suppressed by Rho-ROCK activity, which is required for stress fiber formation. To determine whether FKBP51 influences Rho-mDia or Rho-ROCK signaling, we assessed the formation of membrane ruffles and actin stress fiber by immunostaining with a cortactin antibody and phalloidin-ATTO565, respectively. We observed differences in the formation of membrane ruffles between FLAG-FKBP51-expressing U2OS cells and mock-treated cells (Fig. 1a). FLAG-FKBP51-expressing cells showed decreased membrane ruffles when compared with mock-treated cells. To investigate whether FKBP51 overexpression altered Rho-ROCK activity, ROCK1 protein was fractionated with anion-exchange chromatography (Fig. 1b, c). The expression of FLAG-FKBP5 significantly activated ROCK activity in an immunoblot assay, and this effect was attenuated by a specific ROCK inhibitor Y27632 and a lack of ATP (Fig. 1c, $n = 3$, $P < 0.05$). (Takaoka M. *et al.* Cancer Science 108, 2017)

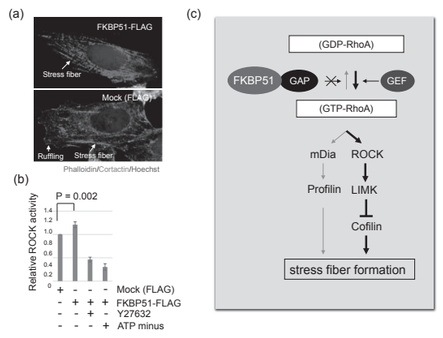

Figure 1. ROCK activity in FLAG-FKBP51-expressing U2OS cells
(a) U2OS cells were transfected with FLAG-FKBP51 or the FLAG-mock expression vector for 24 h. Next, cells were immunostained with anti-cortactin antibody (green) and phalloidin-ATTO565 (red). Nuclei were stained with Hoechst 33258 (blue). (b) The relative ROCK activity with or without the ROCK inhibitor Y27632 (10 μM) or ATP (125 μM) ($n = 3$). (c) The stress fibers is regulated by Rho-ROCK effector pathway via FKBP51.

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Department of Molecular Epidemiology

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Many common chronic diseases are multifactorial in that they are caused by multiple genetic and environmental factors. By applying the information of human genome to epidemiological studies, we aim to clarify the role of genetic polymorphisms, epigenetic changes, as well as their interaction with environmental factors, which contribute to the development of these diseases. We also focus on the mechanism of the Developmental Origin of Health and Disease (DOHaD) hypothesis.

1. Life-course genome study

Many susceptibility tests for common diseases, based on the analysis of common single-nucleotide polymorphisms (SNPs) in individuals, are marketed directly to consumers. Personal genomic testing (PGT) using such tests has long been expected to contribute to the personal motivation to prevent disease. However, recent meta-analyses do not support the hypothesis that DNA-based risk estimates for disease occurrence motivate risk-reducing health behaviors. Existing evidence is unconvincing that DNA-based risk assessments motivate people to take clinical measures that reduce disease risks, such as surgery, even when the subject is at high risk for a disease. These observations may be due to the low clinical validity and utility of DTC-type genomic testing, which have long been criticized, but no consensus exists so far regarding which aspects of a DTC service should jeopardize its permissibility, and consumers are undergoing DTC-type PGT in significant numbers.

While it has been pointed that the explanations attached to the test results produced by commercially available DTC tests usually appear to be insufficient and that DTC-type PGT requires tailored follow-up information to be effective, few studies have focused on the means of delivering information on DTC-type test results.

We therefore conducted planned PGT, taking a detailed family history for each subject, accompanied by a prepared consultation with a physician to examine how this structured testing process would affect the participants thoughts on lifestyle and disease prevention. While this study was conducted as the preliminary stage of a follow-up study of PGT, using a more detailed analysis of each personal genome, including exome sequencing, it aimed to investigate the effectiveness of providing a physician consultation with the delivery of PGT results on participant's perceptions of lifestyle changes and disease-prevention activities.

2. Exome-wide analysis of Abdominal Aortic Aneurysm (AAA)

Abdominal aortic aneurysm (AAA) is a multifactorial disease with strong genetic components. Various genetic loci have been associated with clinical AAA, but few studies have investigated pathological AAA, an intermediate phenotype of the disease. We examined 2263 consecutive autopsies of older Japanese subjects from a study on geriatric diseases in Japanese individuals (The JG-SNP study). The presence of AAA was determined with a pathological diagnosis during autopsy. Single nucleotide variants (SNVs) associated with AAA were determined with an Illumina HumanExome Beadchip array. Logistic regression analyses were performed to determine genetic associations. Age, gender, and other risk factors of AAA were analyzed as covariates. 118 subjects with AAA and 2145 subjects without AAA were analyzed in a case-control setting. No variants reached significance after applying the Bonferroni correction ($P < 2.05 \times 10\text{-}6$). The strongest associations were found with rs3750092 (p.E321G, OR: 0.36, 95% CI: 0.24-0.56, $P = 6.09 \times 10\text{-}6$), a variant in the WAS/WASL interacting protein family 3 (WIPF3), and with rs1051338 (p.T16P, OR: 2.50, 95% CI: 1.70-3.66, $P = 2.79 \times 10\text{-}6$) and rs2246942 (intronic, OR: 2.32, 95% CI: 1.58-3.41, $P = 1.61 \times 10\text{-}5$), variants in the lysosomal acid lipase A (LIPA). LIPA is essential for macrophage cholesterol metabolism. Immunohistological analyses of WIPF3 protein in AAA samples from three subjects revealed that WIPF3 was expressed in macrophages of atheromatous plaques. This study suggests that WIPF3 and LIPA, both of which are expressed in the macrophages are involved in pathological AAA. These results should be regarded as hypothesis-generating; thus, replication study is warranted.

3. Early gestational maternal low-protein diet diminishes hepatic response to fasting in young adult male mice.

Maternal low-protein (MLP) diet can lead to hepatic steatosis, which only develops with ageing. It is still unclear whether the young offspring show any signs of past exposure to prenatal adverse conditions. We hypothesized that early nutritional insult would first affect the dynamic responsiveness to nutritional challenges rather than the static state. We analyzed the transcriptome and metabolome profiles of the hepatic response to fasting/refeeding in young male mice offspring to identify changes induced by early gestational MLP diet. Restricted MLP exposure strictly to early gestation was achieved by the embryo transfer method. As a result, the fasting-induced upregulation of genes related to long-chain fatty acid metabolism and of stress response genes related to protein folding were significantly diminished in MLP pups. Lipid profiling after fasting showed that the hepatic signature of triacylglycerols was shifted to longer acyl-chains and higher saturation by the MLP diet. Bioinformatic analyses suggested that these phenomenological changes may be partially linked to the peroxisome proliferator activated receptor α (PPARα) pathway. Taken together, early gestational MLP diet affected the hepatic dynamic response to nutritional stress in seemingly healthy young offspring, accompanied with partial deterioration of PPARα action.

4. Birth Cohort-Gene ENvironment Interaction Study in TMDU (BC-GENIST)

In order to study the underlying mechanism of Developmental Origin of Health and Disease (DOHaD) hypothesis, we employ a birth cohort in collaboration with the department of Comprehensive Reproductive Medicine at TMDU. We search for epigenetic markers relevant to various phenotypes affecting health conditions both in mothers and babies.

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Department of Genomic Pathology

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Assistant Professor Hiroto Katoh
Assistant Professor Daisuke Komura

Research content

Tumor tissue is a complex system composed of tumor cells and multiple types of stromal cells. It is important for the understanding of developmental mechanisms of the disease to reveal the cell-cell interactions and interferences. Our purpose is to understand the dynamic multicellular interactions in such a complicated biological system by measuring a large amount of data at the genomic level, which leads to the identifications of therapeutic targets and biomarkers.

Research introduction

1. Genomic approach for the cancer-stromal interaction

We have developed a new method to analyze a wide range of cancer-stromal interactions in tumor tissues which are composed of various types of cells (tumor-stroma interactome). This kind of analysis has been technically difficult to be performed comprehensively and quantitatively. By obtaining the transcriptome data of tumor tissues from tumor bearing mouse, we create gene expression profiles of tumor cells (human cells) and stromal cells (mouse cells) by dividing the sequencing reads into human and mouse. Then, we reveal a global picture of the tumor-stroma interactions by incorporating the protein interaction database (Fig. 1). We are going to identify more integrated interaction profiles using this method.
We are trying to reveal a global picture of interactions between cancer cells and stroma by this method and to identify inevitable signaling pathways on which the tumor microenvironments rely. We identified a number of important signals from stroma to cancer cells by using this method in pancreatic cancer xenograft mouse model.

2. Cancer Immunogenomics

Tumor infiltrating lymphocytes (TILs) seem to play important roles in cancer immunity, as suggested by the finding that the amount of TILs correlates with prognosis in various cancer. However, their functions have remained largely unknown. We try to uncover the functions of TILs in cancer environment by analyzing their antigen receptor sequences using massively-parallel sequencing technology.

3. Genomics Analysis for Clinical Disease Tissues

We have been investigating various clinical disease samples by genomics approaches. By utilizing massively-parallel sequencing, we are obtaining comprehensive data of transcriptome and whole exome sequencing of clinical tissue samples and trying to elucidate the pathogenic mechanism of the diseases defined by genomics aspects.

4. Functional Genomics Screening

We are conducting various kinds of functional genomics screening by combining whole genomic shRNA lentivirus libraries and next-generation sequencing technologies. Our goal is to identify novel therapeutic molecular targets against cancers, and to this end we are exploring possible candidate genes by developing a couple of shRNA screening methods. An example of our screening strategies is a tumor implantation model in which various human cancer cell lines infected with whole-genomic shRNA lentivirus library are inoculated into mice. In this model, we can quantitatively characterize the populations of cancer cell clones with each shRNA before and after the tumor implantations. We are now identifying candidate genes which significantly suppress cancer cell growths in vivo. We performed numbers of functional genomics screenings targeting various human cancers, having identified some candidate therapeutic target genes.

5. Image analysis and machine learning in digital pathology

Various genetic abnormalities in cancer cells result in their cellular and architectural abnormalities. Historically, investigating these relationships have provided various insights into cancer biology and gene functions. However, manual investigation of all the relationships in all cancer types by human pathologists is infeasible. We apply deep learning algorithms, which exhibits superior performance in object recognition over conventional machine learning algorithms, to histopathological image analysis of various cancer types. Our aims include inference of clinically relevant somatic mutations from histopathological images, which can be a cost-effective diagnostic tool, and uncovering novel function of genes by investigating the effect of the mutation on the appearance of cancer tissues.

Highlight

1. Immunogenetic Profiling for Gastric Cancers Identifies Sulfated Glycosaminoglycans as Major and Functional B Cell Antigens in Human Malignancies

Gastric carcinoma is one of the most frequent malignancies in Japan. Although anti-tumor immunity has gained substantial attention in recent years because of the clinical success of immune-checkpoint blockade therapies, the efficacy against gastric carcinomas remains elusive. Especially, immune-checkpoint blockade therapies against diffuse-type gastric carcinoma (DGC), which shows the worst prognosis of all forms of gastric carcinoma, may be less effective because DGCs are known to harbor less frequent somatic mutations. In this research we unraveled global landscape of tumoral B cell immunity in diffuse type gastric carcinoma by repertoire sequencing and identified sulfated glycosaminoglycans (GAGs) are major and functional B cell antigens among gastric tumors. Furthermore, natural anti-sulfated GAG antibodies discovered in gastric cancer tissues showed robust growth-suppressive functions against a wide variety of human malignancies of various organs.

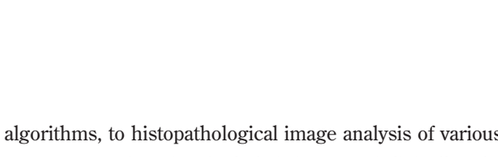

Fig. 1

2. Automated detection and classification of breast cancer metastasis in histopathological images using deep learning

Lymph node metastasis of breast cancer is an important factor affecting the patient's treatment and prognosis. Although this diagnosis is usually made by observing the histopathological tissue using a microscope, small lesions could be overlooked, and the difference in diagnosis by pathologists also becomes a problem. In this research, we trained deep learning using approximately 300,000 images as training data from the cancerous regions and other regions in the histopathological tissue image of the lymph node tissue of breast cancer patients. We efficiently learned the network by extracting characteristic information of the pathological tissue image from the intermediate layer of the neural network, and achieved AUC 0.976 in the image patch level. We further created a probability map of the cancer cells superimposed on the pathological image, and finally evaluated metastasis for each lymph node and the stage of breast cancer patients. We won the 3rd among the participating teams in accuracy. Such a technique could be used for diagnostic aid for breast cancer lymph node metastasis, and in the future, for the pathological diagnosis of various cancers.

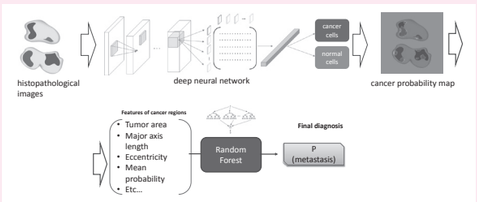

Fig. 2

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Department of Epigenetics

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Introduction of Department of Epigenetics

Epigenetics and Genetics are basics of biology that enables us to elucidate several 'genomic functions' in inheritance, development and evolution of the organisms including our human beings. Genomic imprinting is a mammalian-specific epigenetic mechanism that gives rise to functional differences between paternally- and maternally-derived genomes in development, behavior and growth. Somatic cloned animals give us unique chances to examine 'genetically identical but epigenetically diverged animals'. Mammalian-specific LTR retrotransposon-derived genes are essential for mammalian development, such as placenta and brain functions. These studies show us how Epigenetics and Genetics are important in mammalian biology. We focus on these mammalian-specific genomic functions to elucidate how these genomic functions work and have been evolved as new genomic functions during evolution. Our final goal is to contribute to the human biology as well as medicine in the 21st century by novel understanding of genomic functions.

Research subjects

1. Roles of LTR-retrotransposon-derived genes in mammalian development and evolution

Among sushi-ichi retrotransposon homologue (SIRH) genes, *PEG10/SIRH1* is a therian-specific genes, present in marsupials and eutherians but absent in monotremes while *PEG11/RTL1/SIRH2* and all the other genes are eutherian-specific. We demonstrated that *PEG10, PEG11/RTL1 and SIRH7/LDOC1* are essential for establishing mammalian viviparity and *SIRH11/ZCCHC16, SIRH3/LDOC1L and SIRH8/RGAG4* are important brain genes by a series of knockout mouse analyses.

2. Parental age and gene expression profiles in individual human blastocysts

Physiological environment of the process of gametogenesis, including parental age, may affect the epigenome of the embryo after fertilization. Thus, it is especially important to clarify the influence of parental age on gene expression in the embryo in terms of transgenerational epigenetics to improve the techniques used in assisted reproductive medicine. By performing single embryos RNA-seq analysis on human blastocysts fertilized by intracytoplasmic sperm injection, we identified a number of genes in which the expression levels are lowered with increasing maternal age: some are considered to be important for meiotic chromosomal segregation and others are cytokine important for embryo implantation, suggesting that epigenetic modification of oocyte genome, may change with parental age and be transmitted to the next generation (Sci Rep 2018).

3. Faithful DNA methylation of genomic imprinting in ES cells

ES and iPS cells are useful tools in genetic engineering and regenerative medicine. However, it is not well recognized that DNA methylation memories of genomic imprinting are fragile in these pluripotent cells. Even though they keep their pluripotency for long time but lose imprinting memories in most cases. Here we established a preferable *in vitro* condition for maintaining faithful DNA methylation imprinting in mouse embryonic stem cells (Genes Cells 2018).

Highlight

Why are retrotransposon-derived domesticated genes accumulated in X chromosome?

Several *SIRH* genes derived from long terminal repeat (LTR) retrotransposons played a role in generating mammalian-specific traits, such as a unique viviparous reproductive system and a highly developed central nervous system.

Interestingly, most *SIRH* genes are located on the X chromosome. Is there a specific reason for or advantage of having an X-linked chromosomal location? We propose a novel hypothesis focusing on the role of X chromosome inactivation during this process. The integrated retrotransposons or retrotransposon derivatives are typically harmful rather than advantageous; therefore, it is likely that they behave like dominant negative genes. We previously proposed a hypothesis that in the course of retrotransposon domestication, neutral or nearly neutral evolution preceded Darwinian evolution and helped supply novel materials for novel functional genes from integrated retrotransposons. According to the neutral and nearly neutral theories of molecular evolution proposed by Motoo Kimura and Tomoko Ohta, respectively, neutral or nearly neutral (less harmful) mutations could be fixed in a population by random drift.

In the case of autosomal integration, both males and females would be considerably affected and would exhibit lethality (Fig. 1a). In the case of X chromosomal integration, males would also be lethal. However, some females would have a chance to survive because X-inactivation could make such dangerous inserts less harmful (red dotted circle), whereas others would be lethal. This would depend on which parts of somatic cells in individual were rescued by random X-inactivation because females comprise mixture of two types of the cells in term of X-inactivation (Fig. 1b). As paradoxical as it may seem, it may be also important that all mutant males would die and only normal wild-type males could survive. Then, mutant females would always mate with normal healthy males and would reproduce some viable female mutants and wild-type male offspring from generation to generation (Fig. 1c). In this scenario, even harmful DNA sequences could be stably maintained in a population by transmission through the heterogenous mutant females over a long period, allowing the accumulation of multiple mutations that is needed to generate advantageous genes. Once viable males with such slightly advantageous genes appeared, such genes would be propagated rapidly in both females and males and would finally be fixed in the population. Thus, we propose that X-inactivation in females played a critical role for the accumulation of numerous X-linked *SIRH* genes.

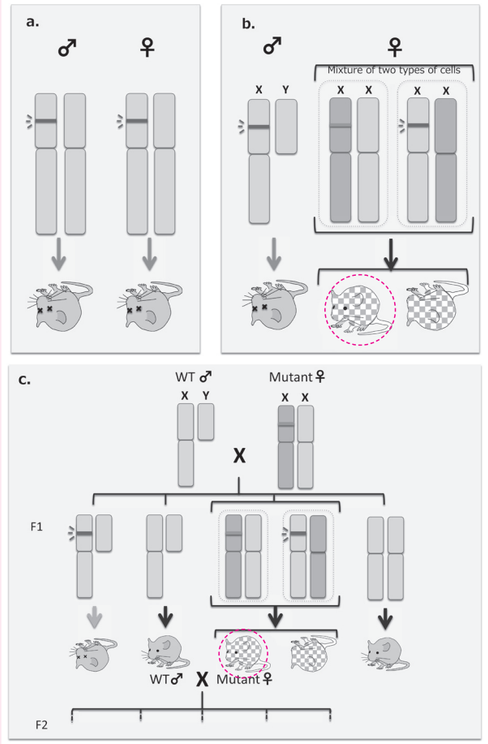

Figure 1. Why are retrotransposon-derived domesticated genes accumulated in X chromosome?
How did X-inactivation work in the domestication of X-linked SIRH genes? Comparison of the results of retrotransposon integration in an autosome (a) and the X chromosome (b). Retrotransposon-derived sequences shown as red bars are hypothesized to function as dominant negative mutations, therefore, would cause lethality in both males and females in most cases when they were integrated in autosomes, whereas harmful effects of the integrated retrotransposon would be reduced by X-inactivation (gray) and some females could be survived (red dotted circle). According to the nearly neutral theory of molecular evolution, a less harmful mutation can be fixed in a population by a random drift mechanism. The integrated retrotransposon would be maintained by transmission from heterogenous mutant females in each generation (c). During long term of evolution, some retrotransposon-derived sequences might acquire mutations that could make them non-lethal for host organisms and/or even advantageous in rare cases. After that, such DNA sequences would become fixed in the population as novel endogenous genes according to the natural selection proposed by Charles Darwin.

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Department of Medical Science Mathematics

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Research Summary

Recently, medical application of rapidly progressing omic profiling technologies and, in particular, the promotion of precision medicine have been keenly desired. Our department overcomes such medical science issues by using a combination of mathematics and computational sciences. Nowadays, biomedical big data of clinical and omic profiles are collected from hospitals and medical institutions. First, applying data-mining methodologies, we explore etiologies of intractable diseases, e.g. cancer, common diseases, and neurodegenerative diseases. Next, we classify each disease into finer categories through molecular profiles, and understand disease causing mechanisms through a systems approach. In this way, we can collect knowledge of disease incidence and progression based on clinical and omic data. Last, we apply mathematical methods, e.g, machine learning techniques, to predict optimized therapy for each patient.

Research Projects

1. Medical Big Data Analysis for Precision Medicine

Toward the goal of personalized/precision medicine, we fully integrate omics, clinical, and molecular datasets into big data. We develop methodologies and utilize advanced statistics and computer science techniques for the analysis of medical big data (Fig. 1a; ***BioData Mining***, 2017; ***BMC Bioinformatics***, 2017a; ***BMC Bioinformatics***, 2017b; ***BMC Bioinformatics***, 2017c; ***IEEE Transactions on Biomedical Engineering***, 2017; ***PLoS One***, 2018; ***BMC Genomics***, 2018; ***Bioinformatics***, 2018; ***Journal of Theoretical Biology***, 2017a; ***Journal of Theoretical Biology***, 2017b; ***Analytical Biochemistry***, 2017). Using these methodologies, we have identified etiologies of rheumatoid arthritis (***Nature Genetics***, 2017), Kawasaki-disease (***Journal of Human Genetics***, 2017), cholesterol and triglyceride levels (***Human Molecular Genetics***, 2017), and kidney functions (***Nature Communications***, 2016), in addition to a significant correlation between the molecular clustering of omic data and clinical information, e.g. survival time in liver cancer omic analysis (***Nature Genetics*** 2016), and intrahepatic metastasis from multicentric tumors (***Journal of Hepatology*** 2017). In addition, we participated in the international consortium for asthma (TAGC), and found five novel loci related to asthma through GWAS meta-analysis (Fig. 1b, ***Nature Genetics***, 2018). These largely overlapped with loci for autoimmune and inflammatory diseases, which suggests roles in regulating the immune system.

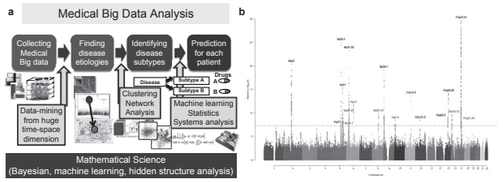

Figure 1 Medical Big Data Analysis for Precision Medicine.
(a) Common analysis steps and methodologies. (b) Newly discovered loci for asthma (***Nature Genetics***, 50, 42-53 (2018)).

2. Investigation of pathogenic mutations for congenital neurological diseases and hearing loss.

We recently established a consortium with the aim of identifying disease-causing mutations and applying that knowledge to clinical diagnosis of congenital neurological diseases and hearing loss between a group of research institutes and hospitals in Japan. To that end, we performed targeted resequencing and whole-exome sequencing (WES) analysis on ~160 families (~500 individuals). We identified some novel pathogenic mutations for the diseases (***Scientific Reports***, 2017; ***BMC Medical Genetics***, 2017; ***International Journal of Opthalmology & Eye Science***, 2017; ***American Journal of Medical Genetics Part A***, 2017a; ***American Journal of Medical Genetics Part A***, 2017b; ***Journal of Human Genetics***, 2017a; ***Journal of Human Genetics***, 2017b; ***Journal of Neurochemistry***, 2017). Also, taking hereditary diabetes cases as an example, we established a method to identify the relationship between mutations in protein domains and the severity of disease through enrichment analysis, and identify associations between the mutations and the protein structures (Fig. 2, ***Diabetes***, 2017). Furthermore, now we are extending our methodologies from WES to whole-genome sequencing (WGS) to explore novel disease-causing genes.

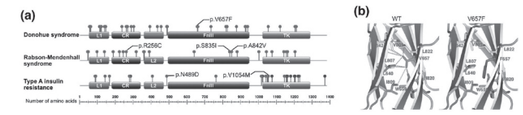

Figure 2 Diabetes causing mutations and protein structure change.
(a) Genomic position of mutations for different levels of disease severity; There is significant enrichment within the Fibronectin type-III domain of insulin receptor (*INSR*) gene in the most severe phenotype, Donohue syndrome. (b) Protein structures differences between wild type (WT) and V657F, which we newly discovered (fig. modified from ***Diabetes***, 2017).

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Frontier Research Unit
Laboratory of Gene Expression

Associate Professor Hidehito KUROYANAGI
Project Assistant Professor Shotaro WANI

Recent whole genome sequence analyses revealed that a high degree of proteomic complexity is achieved with a limited number of genes. This surprising finding underscores the importance of alternative pre-mRNA splicing, through which a single gene can generate structurally and functionally distinct protein isoforms. Based on recent transcriptome analysis, >90% of human multi-exon genes produce multiple mRNA isoforms. Regulation of the splice site choice through so called "splicing codes" provide a versatile mechanism for controlling gene expression and for generation of the proteome diversity. We are trying to decipher the splicing codes in living organisms.

A Fluorescence Reporter System Offers a Path to Alternative Splicing Codes *in Vivo*.

We have developed a transgenic fluorescence alternative splicing reporter system that enables visualization of alternative pre-mRNA processing patterns *in vivo* (Nat Meth, 2006; Nat Protoc, 2010). With this reporter system, we have visualized spatiotemporal profiles of tissue-specific and/or developmentally regulated alternative splicing events in living nematode worms *C. elegans*. By isolating and analyzing mutant worms defective in the color profiles, we have identified *trans*-acting factors and *cis*-elements involved in the splicing regulation (Mol Cell Biol, 2007; Genes Dev, 2008; PLoS Genet, 2012; PLoS Genet, 2013; NAR, 2016; Nat Commun, 2016). We have solved solution structure of two RNA-binding proteins cooperatively recognizing their target RNA stretch by sandwiching a hydrophobic guanine base (Nat Struct Mol Biol, 2014). We recently found that alternative splicing of a tropomyosin gene is differentially regulated in the head muscles and confers a specific function (Mol Biol Cell, 2018). Through these studies, we now realize that molecular mechanisms of the alternative splicing regulation are conserved throughout metazoan evolution (WIREs RNA, 2017).

Global Search for Target Events of Tissue-Specific Splicing Factors.

We are searching for alternative splicing events that are affected in the splicing factor mutants through transcriptome analyses by utilizing a next generation sequencer. We found new target events for a neuron-specific splicing factor UNC-75 and identified its *cis*-elements through bioinformatic and reporter analyses (Nucleic Acids Res, 2013). We have also identified natural non-coding mRNA isoforms that are rapidly degraded by nonsense-mediated mRNA decay (NMD) *in vivo* (NAR, 2016).

Food Bacteria Affect Phenotypes of the Predator Worms.

Commensal bacteria in the gut have recently been shown to affect metabolism, gene expression and physiology of the host animal. We analyzed the effect of genotypes of E. *coli* on the amino acid toxicity to worms *C. elegans* and found that bacterial genes are critical for the toxicity of an amino acid tryptophan (see Figure). This study indicates that bacteria not only serve as a mere nutrient but also function as an important part of predator metabolism. The bacteria-worm system is an excellent model for genetic analysis of the food-predator relationship and will further provide insights into commensalism in higher organisms.

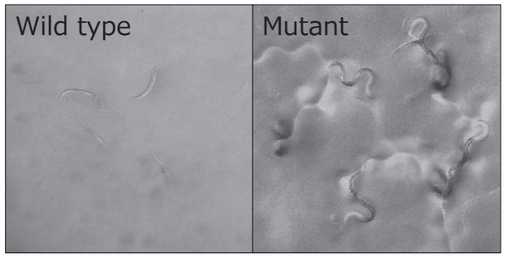

Figure. Bacterial genotypes affect amino acid toxicity to worms.

Publications

Original Articles
Dawn E. Barnes, Eichi Watabe, Kanako Ono, Euiyoung Kwak, Hidehito Kuroyanagi, Shoichiro Ono. Tropomyosin isoforms differentially affect muscle contractility in the head and body regions in the nematode *Caenorhabditis elegans*. Molecular Biology of the Cell (doi : 10. 1091/mbc. E17-03-0152, 2018).

Review Article
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Project Research Unit

Associate Professor Michinori Kubota

Response properties and spatiotemporal organization of multimodal sensory interactions have not been fully understood in the early sensory cortices. To elucidate the characteristics of these interactions in the cerebral cortex, neuronal responses to visual stimuli with or without auditory stimuli were investigated in core and belt fields of guinea pig auditory cortex using real-time optical imaging with a voltage-sensitive dye (RH795). Visual responses consisted of short excitation followed by long inhibition and there were regional and temporal differences in responses. The most salient visual responses were observed in the caudal belt fields, especially posterior (P) and dorsocaudal belt (DCB) fields. Visual responses emerged first in those fields and then spread rostroventrally to core and ventrocaudal belt (VCB) fields. When combined visual and auditory stimuli were applied, fields P and DCB were more inhibited than core and VCB fields. Correspondingly, differences between responses to auditory stimuli alone and combined audiovisual stimuli became larger in fields P and DCB than in core and VCB fields. These data indicate that visual influences are most salient in fields P and DCB, which manifest mainly as inhibition, and that they enhance differences in auditory responses among fields.

Publications

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Kubota M, Sugimoto S, Hosokawa Y, Ojima H,

Laboratory for Integrated Research Projects on Intractable Diseases
Advanced Technology Laboratories

Laboratory for Integrated Research Projects on Intractable Diseases

IBD project, Laboratory for Integrated Research Projects on Intractable Diseases

Professor Shigeomi SHIMIZU
Akinori KIMURA
Toshiaki OHTEKI

Summary

Inflammatory bowel disease (IBD) primarily includes ulcerative colitis and Crohn's disease. Our goal is to understand the mechanism of IBD development and find the new therapies and treatments of the disease.

Research Outcome

1, Involvement of MKL1 in IBD pathogenesis: Because expression of Mkl1 was found to be increased in colonic lamina propria macrophages from DSS-treated mice, a transgenic mouse line (MKL1-Tg) overexpressing human MKL1 specifically in monocyte/macrophage cell lineage was established. MKL1-Tg spontaneously displayed IBD phenotypes and showed higher susceptibility to DSS-induced colitis, indicating that MKL1 crucially contributes to the development of colitis via regulating function of macrophages (ref.1)

2, Identification of human common monocyte progenitor (cMoP) cells: In this paper, we identified human cMoPs as a CLEC12A^{hi}CD64^{hi} subpopulation of conventional granulocyte-monocyte progenitors (cGMPs). Human cMoPs gave rise to monocyte subsets without showing any potential for differentiating into myeloid or lymphoid cells. Within the cGMP population, we also identified revised GMPs that completely lacked DC and lymphoid potential (ref.2).

3, Crucial role of autophagy in intestinal stem cell (ISC) maintenance: In this paper, we generated intestinal epithelial cell-specific Atg5-deficient mice (*Atg5*$^{\Delta IEC}$ mice) and showed the crucial role of autophagy in ISC homeostasis, because these mice had significantly fewer ISCs and showed impaired ISC-dependent intestinal recovery after irradiation. We also found that a ROS-inducing reagent decreased the ISC number and impaired their regenerative capacity *ex vivo*, and treating *Atg5*$^{\Delta IEC}$ mice with an antioxidant rescued their defects (ref.3)

4, Analyses of pathogenesis of DSS colitis: Using a DSS induced colitis model, we showed that accumulated Ly6C^{+} cells consisting of inflammatory monocytes and inflammatory macrophages strongly expressed representative colitogenic mediators such as TNF-α and iNOS. The IFN-γ–Stat1 pathway was required for generating colitogenic macrophages, given that *Stat1*$^{-/-}$ mice had less severe colitis and fewer colitogenic macrophages. Notably, IFN-γ induced histone acetylation at the promoter regions of the *Tnf* and *Nos2* loci in the monocyte and macrophage lineage, indicating that IFN-γ–dependent epigenetic regulation instructs the development of the colitogenic monocyte and macrophage lineage *in vivo*. Collectively, our results provide the essential mechanism by which dysregulated colitogenic monocytes/macrophages develop at the colon mucosa during inflammation.

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Research Project on Striated Muscle Diseases

Project Leader, Molecular Pathogenesis Akinori KIMURA
Gene Expression Hidehito KUROYANAGI, Shotaro WANI
Cellular and Molecular Medicine Yumiko OISHI, Shinichiro HAYASHI
Molecular Pathogenesis Jianbo AN, Taeko K. NARUSE

Heart-Specific Splicing of the *TTN* Gene in Dilated Cardiomyopathy.

Dilated cardiomyopathy (DCM) is a heart disease characterized by left ventricular dilatation and systolic dysfunction. DCM can be caused by mutations in sarcomere protein genes including *TTN*. The *TTN* gene consists of 363 exons and its pre-mRNA splicing pattern is developmentally regulated and varies between cardiac muscles and skeletal muscles. An RNA-binding protein RBM20 is shown to be a major regulator of heart-specific alternative pre-mRNA splicing of *TTN*. Mutation in RBM20 is linked to autosomal-dominant familial DCM, yet most of the RBM20 missense mutations were mapped to an RSRSP stretch whose function remains unknown. We constructed a fluorescence reporter minigene to successfully visualize the heart-specific splicing regulation of the *TTN* gene. By utilizing this reporter, we found that the two serine residues in the RSRSP stretch are critical for nuclear localization of RBM20. We also found that the residues are constitutively phosphorylated in cells. Furthermore, we identified an S634W missense mutation within the RSRSP stretch in a DCM patient, which also affected nuclear localization of RBM20. *Rbm20*^{S637A} knock-in mouse showed a remarkable effect on titin isoform expression. These study revealed the function of the RSRSP stretch as a critical part of a nuclear localization signal and offer the *Rbm20*^{S637A} mouse as a model for DCM.

Hypoxic Breast Cancer Project

Principal Researcher Koh Nakayama, Ph.D.
Collaborators Ryo Yonashiro, Ph.D.
Hiroshi Shibuya Ph.D.
Yoshio Miki, M.D., Ph.D.
Fumitoshi Ishino, Ph.D.

Research Subject

Tumor microenvironment is often hypoxic, and induces malignant transformation of cancer cells. Breast cancer is one of the major cancers in women worldwide. Thus, it is important to understand how hypoxic environment affects the character of breast cancer. We have been focusing on acute and chronic phases of hypoxic responses, and identified mechanisms to regulate transcription and metabolism under such conditions. In this project, we aim to understand the regulatory mechanism of gene expression in breast cancers which is mediated by epigenetic changes. We currently analyze DNA demethylating enzyme TETs. TETs mediate gene expression by altering the methylation status in cells. Hypoxic regions are known to be formed in breast tumors, and DNA methylation is also reported to be increased in breast cancers. However, it is not clear if hypoxia has anything to do with the methylation status in breast cancer. Thus, we try to address this question by combining approaches based on hypoxic and epigenetic studies (Figure). Our final goal is to establish a new technology to detect an early stage of breast cancer by using the molecules identified in this project.

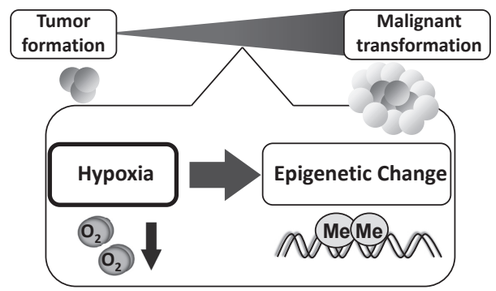


Figure. Scheme of Hypoxic Breast Cancer project

DOHaD research towards preventive and pre-emptive approach against chronic intractable disease

Principal researcher　Noriko Sato
Molecular Epidemiology　Katsuko Sudo, Chihiro Imai
Comprehensive Reproductive Medicine　Naoyuki Miyasaka
Periodontology　Yu-ichi Izumi, Sayaka Katagiri
Epigenetics　Takashi Kohda

Summary

Developmental Origin of Health and Disease (DOHaD) is the concept that the process through which the environment encountered before birth and/or infancy shapes the long-lasting bodily function and physiology. Epidemiological studies have shown that inadequate conditions in early stage of life can predispose us to lifelong diseases, which are developed later. It is crucial to accumulate genomic and epigenomic epidemiological data of prospective birth cohorts, and to analyze how environment interacts with fetal genome and modulates its phenotype, which is still a burgeoning field of research in Japan. Our Birth Cohort – Gene and ENvironment Interaction Study of TMDU (BC-GENIST) will sort out the current environmental conditions possibly threatening mother and child health. We will identify the interindividual epigenetic differences caused by the interaction between the genetic polymorphisms and environmental variables. In order to elucidate the molecular mechanisms how the prenatal conditions form the future disease phenotype, animal models are being investigated. Particularly, we are currently studying the effects of parental ageing on embryo development and the effects of periodontal disease on pregnancy and metabolism.

Research Project

1. More than 70 BC-GENIST participants have delivered babies (by Jan. 2018). The inter-individual variations in DNA methylation in blood cells are being analyzed. In order to elucidate their association with various environmental factors, we are currently preparing the reference data of each blood cell type including nucleated red blood cells (nRBC) existing in cord blood cells.
2. Our animal experiments have shown that (1) suboptimal nutrition in early gestation impairs the hepatic nutritional stress response in the offspring and (2) periodontal disease affects hepatic metabolism and could impair intrauterine fetal growth.

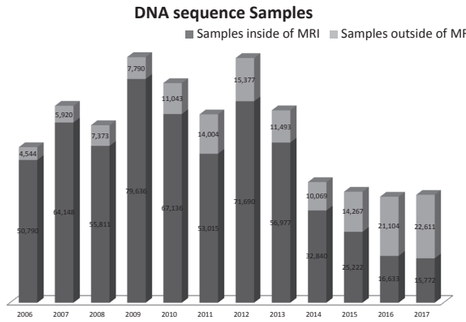
Publications

1. Sato N, Sudo K, Mori M, Imai C, Muramatsu M, Sugimoto M: Early gestational maternal low-protein diet diminishes hepatic response to fasting in young adult male mice. Sci Rep. 2017; 7 (1): 9812
2. Komazaki R, Katagiri S, Takahashi H, Maekawa S, Shiba T, Takeuchi Y, Kitajima Y, Ohtsu A, Udagawa S, Sasaki N, Watanabe K, Sato N, Miyasaka N, Eguchi Y, Anzai K, Izumi Y. Periodontal pathogenic bacteria, Aggregatibacter actinomycetemcomitans affect non-alcoholic fatty liver disease by altering gut microbiota and glucose metabolism. Sci Rep. 2017 ; 7(1):13950.
3. Udagawa S, Katagiri S, Maekawa S, Takeuchi Y, Komazaki R, Ohtsu A, Sasaki N, Shiba T, Watanabe K, Ishihara K, Sato N, Miyasaka N, Izumi Y. Effect of Porphyromonas gingivalis infection in the placenta and umbilical cord in pregnant mice with low birth weight. Acta Odontol Scand. In press

Advanced Technology Laboratories

Genome Laboratory

This laboratory is established to support the education and research in the Medical Research Institute and Graduate School of Biomedical Science. Sequencing analyses requested by researchers belonging to the Medical Research Institute and those to the other Departments of Tokyo Medical and Dental University are done in this laboratory. In addition, several equipment including Flow Cytometer and Plate Reader are under management of the laboratory. Followings are the achievements in 2017. This year we have started cooperating with Research Core belonging to Institute of Research TMDU.



1. Sequencing analyses

A total of 38,383 samples from 3,356 researchers were sequenced in the year of 2017. Among them 22,611 (58.9%) samples were requested by researchers outside the medical Research Institute (see below). Analysis by using next generation sequencing equipment (Ion Torrent PGM) has been started in 2013 and 27 runs were done in the year of 2017.　Library preparation service for next generation sequencing has been started in 2015 and 117 samples were done in the year of 2017.

2. Equipment under the management of the Genome Laboratory.

DNA sequencer (ABI3130xl) × 2, Next generation sequencer (Ion Torrent PGM), PCR machine (ABI7900) × 5, Flow Cytometer, Luminescent Plate Reader, Ice Machine, Centrifuge, Ion One Touch system, DNA/RNA shearing system, and others.

3. Introductory seminars

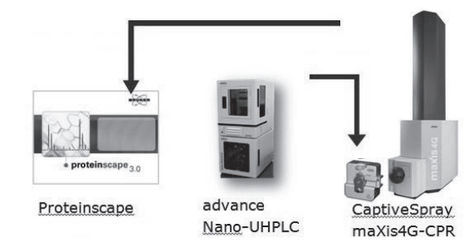
Introductory seminars were done for use of　instruments (2times).

Laboratory of Cytometry and Proteome Research

This laboratory will help researchers to separate specific cells and analyze the proteome expressed in the cells. We set up equipments written below.

For proteome analysis, we have a 2D-electrophoretic system, an LC-MSMS system and a Biacore system in this laboratory. We can accept consignment analysis of proteins with 2D-electrophoresis and the mass spectrometry by request of researchers in this university. In addition, we can provide technical advices on cytometry and proteome researches to young scientists who wish to start their own research.

From 2017, we belong to RCC (Research Core Center) and Nanken-Kyoten in TMDU.



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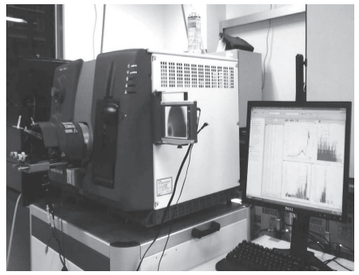
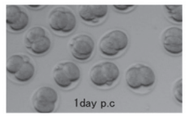


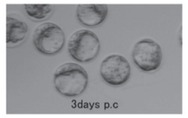
Qtrap5500　ABSCIEX

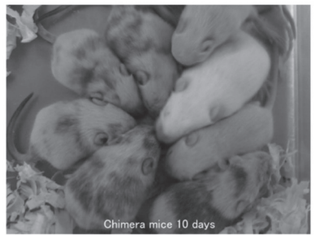
Laboratory of Recombinant Animals

Recombinant animals including transgenic, knock-out and knock-in mice are now essential for the medical and biological studies and education in graduate schools. Those animals are also essential for the research on the pathogenesis and pathophysiology of intractable diseases, and development of new treatments for these diseases. In the Recombinant Animal Laboratory, excellent technical staffs generate, clean up, maintain and store recombinant mice to facilitate the biomedical research in Medical Research Institute. In FY 2015, using genome editing technology, we have started a new support service to generate recombinant animals for TMDU researchers.

Medical Research Institute runs this laboratory as a part of the core facility. The committee of this laboratory composed of faculty members of Medical Research Institute regulates the laboratory by educating new users, and controling generation and introduction of new recombinant mice.







Laboratory of Anatomy and Cell Function

Laboratory of anatomy and cell function (LACF) has a support system for histological technologies at Medical Research Institute (MRI). We equip basic technical instruments and materials for analyzing research of cells and tissue available for common use. LACF provides technological assist for all research laboratories at MRI as shown in the following list and facilities for study.

Website: http://www.tmd.ac.jp/mri/lacf/index.html

<<Common equipment>>

・Confocal laser microscope
… LSM710, LSM510META (Carl Zeiss)
・Cryostat … CM3050s (Leica)
・Rotary microtome … HM-325E, HM-335E (Microm)
・Vibrating microtome … PRO7 (D.S.K.)
・Automated Tissue Processor
… RH-12DM (Sakura Finetek)
Excelsior ES (Thermo Scientific)
・Tissue-embedding-station
… Histostar (Thermo Scientific)
・Real-time PCR … 7500, 7900HT (Applied Biosystems)
・Laser microdissection … LMD7000 (Leica)
・Stereo microscope … SZX-16 (Olympus)

<<seminars>>

A user of Confocal laser microscope is required to attend a seminar for learn of the correct way to use.
In this fiscal year, seminars were done twice (5/26 and 10/23).

Laboratory for Structure Analysis

The Laboratory for Structure Analysis is equipped with a high-brilliancy X-ray generator and an image plate X-ray detector for the structure determination of biological macromolecules. The Laboratory is also equipped with a dynamic light scattering (DLS) instrument, enabling the measurements of the particle size (thus the oligomeric state and aggregation) of protein molecules. An introductory practical course was held for students of Graduate School. The laboratory cooperates with the Joint Usage/Research Program of the Institute (Nanken-Kyoten) and is open for users from the outside of the university.

Stem Cell Laboratory

In order to assist researchers working on stem cell-related subjects across Medical Research Institute, Stem Cell Laboratory was established as of December, 2009. Cooperation with a research core center of this university has started from the current year. Now the Laboratory is equipped with basic and state-of-the-art research instruments, including high-speed cell sorters (MoFlo Legacy and MoFlo XDP), confocal laser scanning microscopes (FV10i-W for time-lapse images, and FV10i-DOC for one shot images).

This Laboratory is managed by the Operating Committee composed of four Professors and two Associate Professors in the Institute, and the services are provided by one Technical Staff and one Technical Assistant.

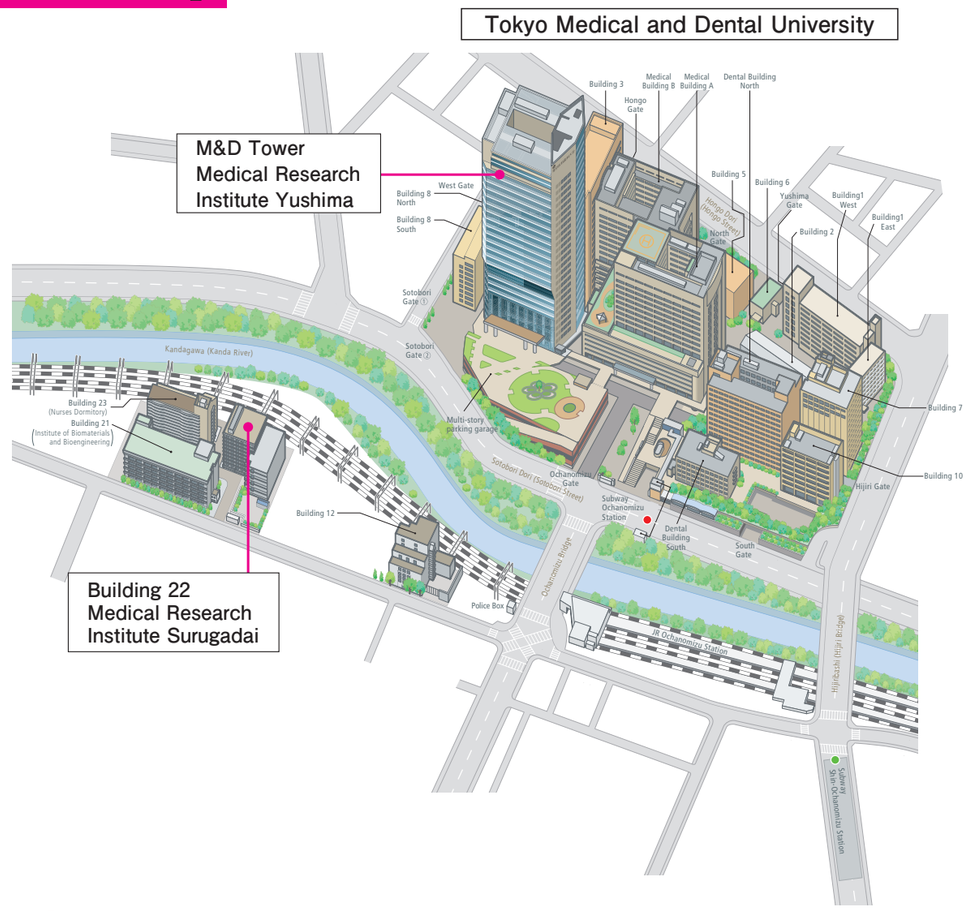
The use of the equipment and cell sorting service are available to researchers in other departments within the University and those outside since August 1st,2013. The number of users is increasing every year.

The number of overall use cases was 521 in the year of 2017. We held 3 short courses for beginners to help them use the equipment.

Advisory Committee Members

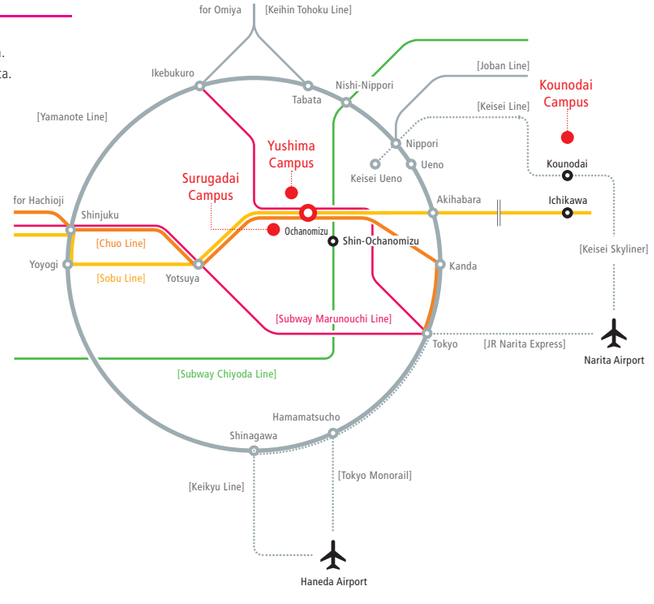
GO Mitiko	Trustee Nagoya University
SASAZUKI Takehiko	University Professor Kyushu University
TANAKA Takaharu	President Hoshi University
TANIGUCHI Masaru	Special Advisor RIKEN Center for Integrative Medical Sciences
NAGAI Ryozo	President Jichi Medical University
NAKAGAMA Hitoshi	Director National Cancer Center Research Institute
NAGANO Tetsuo	Vistiting/Emeritus Professor Drug Discovery Initiative The University of Tokyo
NISHIKAWA Shin-ichi	Advisor JT Biohistory Research Hall

Access Map



Nearest Stations

- JR Line Ochanomizu Sta.
- Subway Marunouchi Line Ochanomizu Sta.
- Subway Chiyoda Line Shin-Ochanomizu Sta.



ANNUAL REPORT 2018

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