Annual Report
2021

ANNUAL REPORT 2021

Tokyo Medical and Dental University

MRi Medical Research Institute | **TMDU**

Medical Research Institute Tokyo Medical and Dental University

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

Tel +81-3-5803-4504

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Medical Research Institute

Tokyo Medical and Dental University

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Division of Advanced Molecular Medicine

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Yushima Area

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

Medical Research Institute

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Surugadai Area

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

Medical Research Institute

Laboratory of Genome Editing for Biomedical Research

Medical Research Institute

Director — **Deputy Director**
Hiroshi Nishina — Shigeomi Shimizu

— Faculty Meeting

Research Divisions

Division of Advanced Molecular Medicine
Department	Title	Name
Department of Molecular Medicine and Metabolism	(under consideration)	
Department of Medical Chemistry	Professor	Katsumori Segawa
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	(under consideration)	

Division of Pathophysiology
Department	Title	Name
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	(under consideration)	

Division of Medical Genomics
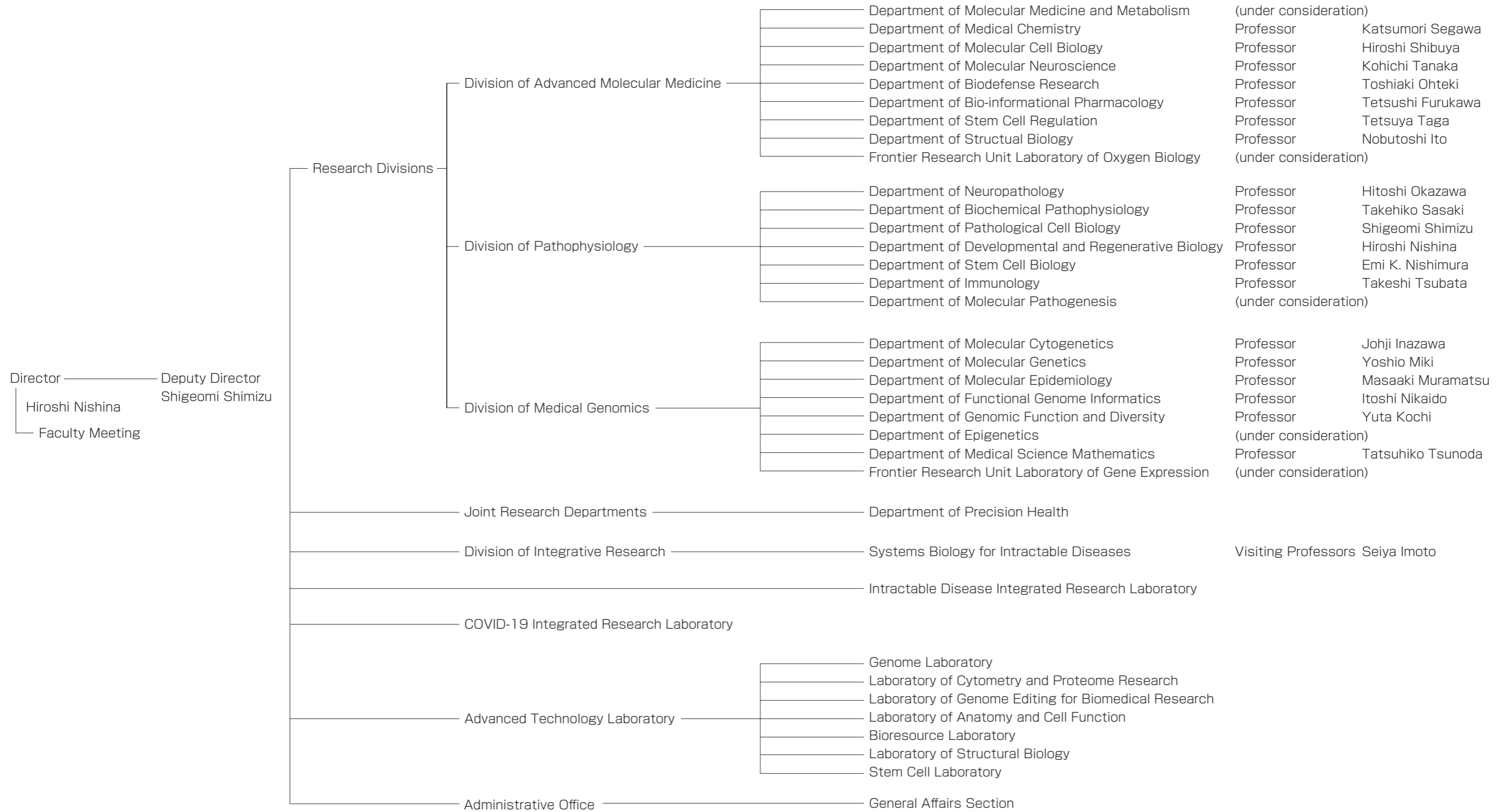| Department	Title	Name
Department of Molecular Cytogenetics	Professor	Johji Inazawa
Department of Molecular Genetics	Professor	Yoshio Miki
Department of Molecular Epidemiology	Professor	Masaaki Muramatsu
Department of Functional Genome Informatics	Professor	Itoshi Nikaido
Department of Genomic Function and Diversity	Professor	Yuta Kochi
Department of Epigenetics	(under consideration)	
Department of Medical Science Mathematics	Professor	Tatsuhiko Tsunoda
Frontier Research Unit Laboratory of Gene Expression	(under consideration)	

Joint Research Departments — Department of Precision Health

Division of Integrative Research — Systems Biology for Intractable Diseases — Visiting Professors Seiya Imoto

Intractable Disease Integrated Research Laboratory

COVID-19 Integrated Research Laboratory

Advanced Technology Laboratory
- Genome Laboratory
- Laboratory of Cytometry and Proteome Research
- Laboratory of Genome Editing for Biomedical Research
- Laboratory of Anatomy and Cell Function
- Bioresource Laboratory
- Laboratory of Structural Biology
- Stem Cell Laboratory

Administrative Office — General Affairs Section

Inter-University Research Network for Trans-Omics Medicine

Since April, 2016, Medical Research Institute at Tokyo Medical and Dental University has been promoting the "Inter-University Research Network for Trans-Omics Medicine Project" aiming to establish a trans-omics research education hub. We have been carrying out this project in cooperation with Kyushu University, Kumamoto University, Tokushima University's Joint Usage/ 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 (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)
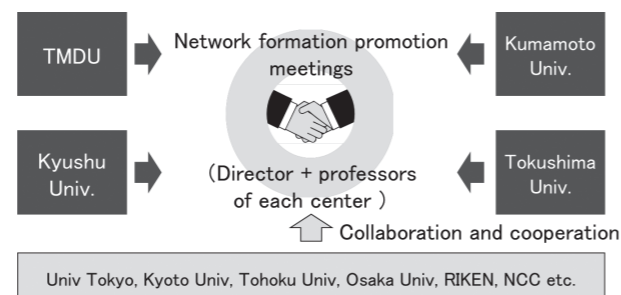
Activities

* Joint research symposium

The 5th Symposium of the Inter-University Research Network for Trans-Omics Medicine

The Future of Trans-Omics in the Age of COVID-19
 (Organized by Medical Research Institute, Tokyo Medical and Dental University)
　Date: January 22, 2021
　Place: Online symposium at ZOOM

Speakers from MRI, TMDU:
Fumitoshi Ishino (Professor, Department of Epigenetics)
　Beyond genomic imprinting: mammalian-specific genes from LTR retrotransposons and primate-specific genes from retroviruses.

Itoshi Nikaido (Professor, Department of Functional Genome Informatics)
　High-throughput transcriptome methods for human cell atlas.

* Technical seminar

The 4rd Trans-Omics Medicine Trans-Omics Medicine Technical Seminar

Date: January 27, 2021
　Place: Online seminar at ZOOM

Lecturers:
Ichiro Hiratani (Team Leader, Center for Biosystems Dynamics Research, RIKEN)
　Introduction of scRepli-seq that enables whole-genome DNA replication analysis from a single cell.
Takashi Kohda (Professor, Faculty of Life and Environmental Sciences, University of Yamanashi)
　Comprehensive analysis technology for cytosine modification of genomic DNA: EnIGMA-seq.
Ayumi Matsuzawa (Assistant Professor, Medical Research Institute, Tokyo Medical and Dental University)
　Detecting the in vivo expression sites of proteins encoded by low expression genes.

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 nor 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, Medical Research Institute (for intractable disease research) 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.
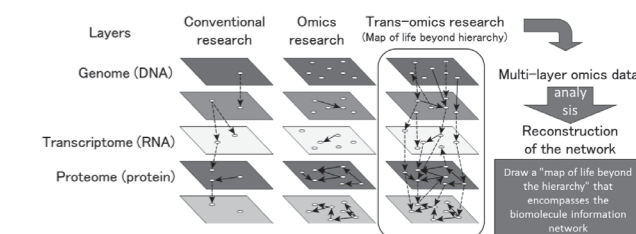
Division of Advanced Molecular Medicine

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】

- The WNK kinase, which is involved in blood pressure regulation, regulates Wnt signaling via the E3 ligase, MAEA / RMND5A.
- WNK is a previously unrecognized regulator of β-Catenin and a therapeutic target of cancer.

【Molecular Neuroscience】

- Glial glutamate transporter GLT1 determines susceptibility to spreading depression in the mouse cerebral cortex.
- Identification of EAAT1 variants associated with glaucoma
- Development of in vivo genome editing using AAV-CRISPR system
- Establishment of a tTA-dependent photoactivatable Cre recombinase knock-in mouse model for optogenetic genome engineering

【Biodefense Research】

- Identification of a new molecular guardian of intestinal stem cells
- Identification of the major cell of origin for regeneration in the damaged mouse intestine
- Development of a new analysis method for stress-induced hematopoietic response

【Bio-informational Pharmacology】

- We identified novel mechanism of development of dilated cardiomyopathy by mutant RNA binding protein.
- We identified a key factor for specifying cardiac progenitor cells from mesenchymal stem cells.
- We discovered epigenetic transcription regulation of inflammation-related genes in vascular endothelial cells.

【Stem Cell Regulation】

- Elucidation of the involvement of transcription factor Sox17-mediated GIMAP6 expression in the hematopoietic capacity in intra-aortic hematopoietic cell clusters
- Elucidation of the mechanisms underlying glioma recurrence by which cancer stem cell (CSC)-derived autoschizis-like products induce tumor-promoting M1-like macrophages

【Structural Biology】

- The interactions between T cell regulatory receptors and signal transduction molecules were revealed through high-resolution crystal structures.
- New lead compounds against tauopathy such as Huntington's disease were found.

Department of Molecular Cell Biology

Professor   Hiroshi Shibuya
Associate Professor   Toshiyasu Goto
Assistant Professor   Masahiro Shimizu

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/threonine 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). 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.

WNK is also involved in developmental and cellular processes. Previously, we found that WNK signaling is involved in the morphological and neural development via Lhx8 and GSK3β. However, more functions of WNK in many cellular processes is predicted. We focus on WNK functions in signal transduction network, especially in Wnt signaling pathway.

1. WNK is a positive regulator of the Wnt signaling pathway

To analyze WNK functions in Wnt signaling, we examined whether knockdown of WNK affects the Wnt signaling pathway, and found that the knockdown of both WNK1 and WNK4 (WNK1/4) using siRNA significantly reduced the induction of Wnt target gens by Wnt stimulation. This suggests that WNK is a positive regulator in the Wnt signaling pathway.

We next analyzed the relationship between WNK and β-Catenin in the Wnt signaling pathway. The knockdown of WNK1/4 reduced the β-Catenin level under both normal conditions and Wnt-stimulated conditions, suggesting

WNK regulates the β-Catenin level.

β-Catenin is phosphorylated by GSK3β in the destruction complex and is ubiquitinated by βTrCP E3 ubiquitin ligase. Thus, we next checked whether β-Catenin degradation caused by WNK1/4 knockdown was mediated by βTrCP. Interestingly, the reduced β-Catenin level could not be rescued by knockdown of βTrCP. This suggests that WNK is not related to the degradation of β-Catenin by the destruction complex containing βTrCP.

2. WNK regulates the protein level of β-Catenin through the GID complex

Our previous report suggests that WDR26, which is contained in GID E3 ligase complex, is involved in the ubiquitination of β-Catenin. Thus, we examined effect of two E3 ligases MAEA and RMND5A, which are components of the GID complex, on WNK-mediated β-Catenin degradation. The reduction of β-Catenin by the knockdown of WNK1/4 could be rescued by the knockdown of both RMND5A and MAEA, suggesting that GID complex is involved in degradation of β-Catenin associated with WNK function. Interestingly, WNK interacts with MAEA and WNK binding attenuates interaction between β-Catenin and MAEA. These data suggest that the presence of WNK inhibits the degradation of β-Catenin by the GID complex.

3. The WNK inhibitor functions as an inhibitor of Wnt signaling and suppresses xenograft tumor growth of colorectal cancer cells

Previous research identified a compound STOCKS2S-26016 (26016) as inhibitor of the WNK signaling pathway. we checked effect of this inhibitor on Wnt signaling, and found that 26016 reduced the β-Catenin level and the expression of Wnt target genes. Furthermore, treatment with 26016 increased the ubiquitination level of β-Catenin. We also analyzed effect of derivatives of 26016, called #13, on Wnt signaling, and similar results were observed. These results suggest that the WNK inhibitor 26016 and #13 functions as an inhibitor of the Wnt signaling pathway, similar to the effect of WNK knockdown.

The induction of β-Catenin degradation by small molecules is effective to suppress colorectal cancer development, suggesting that β-Catenin degradation mediated by the WNK inhibitors 26016 and #13 would repress colorectal cancer development. Therefore, we evaluated the anti-tumor effect of WNK inhibitors. As a result, 26016 effectively induced cell death, and #13 caused significant suppression of cell growth of colorectal cancer cells. We next analyzed the effects of 26016 and #13 on xenograft tumor formation in vivo. Surprisingly, we observed little or no effect of 26016 on xenograft progression at low doses, but at a high dose of 26016, four of five mice died after only two injections. This suggests that 26016 has more severe toxicity than efficacy *in vivo*. By contrast, treatment of #13 resulted in reduced tumor size and weight in a dose-dependent manner compared with the findings for tumors from vehicle injection. Moreover, the amount of β-Catenin in the tumors treated with #13 was reduced in a manner dependent on the concentration of #13 and tumor size. These findings suggest that #13 has low toxicity and prevents colorectal cancer development through β-Catenin degradation.

In conclusion, we demonstrated that WNK is a positive regulator of the Wnt signaling pathway. WNK attenuates the interaction between β-Catenin and the GID complex, suggesting that WNK might regulate β-Catenin levels. Furthermore, we showed that the WNK inhibitor also functions as a Wnt inhibitor, suppressing xenograft tumour development in mice. These findings suggest that WNK is a regulator of β-Catenin and might be a therapeutic cancer target.
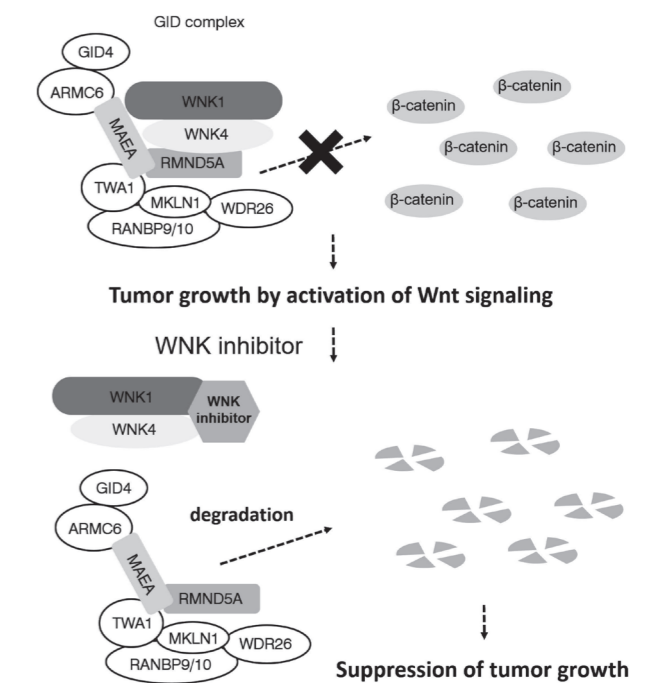
# Department of Molecular Neuroscience

Professor	Kohichi Tanaka
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.

Migraines affect millions of people worldwide, often lasting days and severely disrupting lives. More than simply super-intense headaches, some migraines actually result from pathological excitation of neurons in the brain. We show that susceptibility to migraines could be related to a molecular transporter that normally works to prevent excessive excitation of neurons. Migraines are related to a condition called cortical depression, in which a large wave of hyperactivity spreads across the brain, followed by a wave of inhibition, or depressed brain activity. We hypothesized that susceptibility to cortical spreading depression (CSD) is related to disrupted transport of glutamate, the most common excitatory neurotransmitter. We found that when mice lacked the GLT-1 transporter, cortical spreading depression occurred more frequently and spread more quickly than in control mice or in the other knockout mice. If GLT-1 proves to be disrupted in people who have migraines, drug therapy that acts to
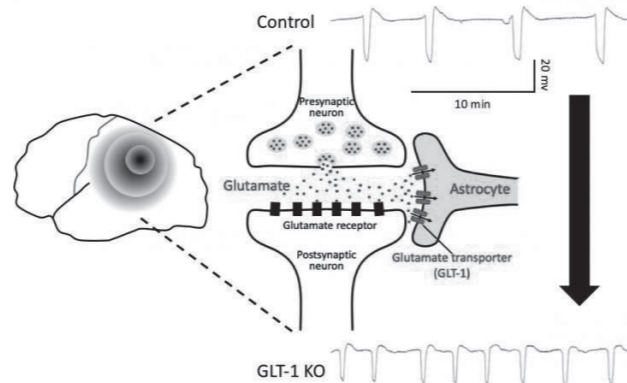
Representative raw traces of the direct current potential changes during CSD in control and GLT-1 knockout mice.

increase glial reuptake of glutamate could be a reasonable therapeutic approach.

2. In vivo genome editing using AAV-CRISPR system

Genetically modified animals play critical roles in understanding neuronal development, function and disease. Conventional methods to establish transgenic animals have been a time- and labor-intensive process. To overcome these limitations, we developed a viral vector-mediated gene knock-out strategy using CRISPR/Cas9. Using this method, we found that a dopamine receptor D1R ablation in the nucleus accumbens (NAc) region effectively increased the active coping behavior in animals under stress, suggesting that the reduced dopamine release in the NAc region initiated an active coping behavior.

Publications

[Original papers]

1. Onodera, M., Mayer, J., Furukawa, K., Hiraoka, Y., Aida, T., Tanaka, K., Tanaka, K.F., Rose, C.R., Matsui, K. Exacerbation of epilepsy by astrocyte alkalization and gap junction uncoupling. *J. Neurosci* (in press)

2. Shin, T., Hiraoka, Y., Yamasaki, T., Marth, J.D., Penninger, J.M., Kanai, M., Tanaka, K., Kofuji, S., Nishina, H. MKK7-deficiency in mature neurons impairs parental behavior in mice. *Genes Cells* 26. 5-17, 2021.

3. Tsuru H., Osaka M., Hiraoka Y. & YoshidaM., HFD-induced hepatic lipid accumulation and inflammation are decreased in Factor D deficient mouse, *Sci. Rep.* 10. 17593, 2020.

4. Ihara K., Sasano T., Hiraoka Y., Togo-Ohno M., Soejima Y., Sawabe M., Tsuchiya M., Ogawa H., Furukawa T. & Kuroyanagi H., A missense mutation in the RSRSP stretch of Rbm20 causes dilated cardiomyopathy and atrial fibrillation in mice, *Sci. Rep.* 10. 17894, 2020.

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7. Cui, W., Aida, T., Ito, H., Kobayashi, K., Wada, Y., Kato, S., Nakano, T., Zhu, M., Isa, K., Kobayashi, K., Isa, T., Tanaka, K., Aizawa, H. Dopaminergic signaling in the nucleus accumbens modulates stress-coping strategies during inescapable stress. *J Neurosci* 40, 7241-7254, 2020.

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10. Aihara, Y., Fukuda, Y., Takizawa, A., Osakabe, N., Aida, T., Tanaka, K., Yoshikawa, S., Karasuyama, H., Adachi, T. Visualization of mechanical stress-mediated Ca²⁺ signaling in the gut using intravital imaging. *Biosci Microbiota Food Health* 39. 209-218, 2020.

11. Aizawa, H., Sun, W., Sugiyama, K., Itou, Y., Aida, T., Cui, W., Toyoda, S., Terai, H., Yanagisawa, M., Tanaka, K. Glial glutamate transporter GLT-1 determines susceptibility to spreading depression in the mouse cerebral cortex. *Glia* 68. 2631-2641, 2020.

12. Takao, T., Hiraoka, Y., Kawabe, K., Yamada, D., Ming, L., Tanaka, K., Sato, M., Takarada, T. Establishment of a tTA-dependent photoactiveatable Cre recombinase knock-in mouse model for optogenetic genome engineering. *Biochem. Biophys. Res. Commun.* 526. 213-217, 2020.

Fig.1 A new analysis method for stress-induced hematopoietic response

Fig.2 Importance of IFN signal suppression in intestinal stem cell maintenance

Department of Biodefense Research

Professor	Toshiaki Ohteki, DDS, Ph.D.
Associate Professor	Taku Sato, Ph.D.
Assistant Professor	Masashi Kanayama, Ph.D.
Adjunct Lecturer	Nobuyuki Onai, Ph.D.
Adjunct Lecturer	Yasuhiro Murakawa, Ph.D.
Restart Postdoctoral Fellowship (JSPS)	Mihoko Kajita, Ph.D.
Research Technician	Kisho Shiseki
Research Technician	Toyoki Hayashi
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 **myeloid cells** (dendritic cells and macrophages), **tissue stem cells**, and their functional interplay in the immunological and non-immunological organs. 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 myeloid cells

1) Identification of novel sources of dendritic cells and macrophages

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) in response to viral and self-nucleic acids. We have discovered the DC progenitors in the mouse bone marrow, and named common DC progenitors (CDPs) (*Immunity* 2013; *Nat Immunol* 2007). Interestingly, we found that 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, were identified in the mouse bone marrow and spleen by other group in 2013. Based on the achievements in mouse, we succeeded to identify human cMoP (*Immunity* 2017; *Int Immunol* 2018). Human cMoP gives rise to only monocytes but not other hematopoietic cells including DCs. Given that monocytes are involved in chronic myelomonocytic leukemia (CMML) and monocyte-derived tumor-associated macrophages (TAMs) promote tumor development, we, in collaboration with a pharmaceutical company, have generated an antibody-drug conjugate that selectively targets human cMoP. Using patient-derived xenograft (PDX) models, we are currently testing the effects of this reagent on CMML and TAM depletion (submitted).

2) Impairment of brain function by microglial enhancer in aging and Alzheimer's disease (AD)

The decline in tissue regeneration and homeostasis associated with life-stage progression is closely related to the functional alteration of macrophages. Microglia, a macrophage in the brain, is actively contributing to the brain development and maintenance during young age (regenerative microglia). However, with age, microglial inflammatory trait becomes prominent with impaired phagocytosis and brain-derived neurotrophic factor (BDNF) production etc (inflammatory microglia). As a result, functional neurons and synapses are decreased and destroyed. However, the overall picture and entire process of the microglial functional alteration and causative epigenomic transformation have not been clarified.

Using a novel RIKEN technology that can detect the active enhancer region at single base level, we are trying to identify the enhancers responsible for the microglial transformation during life-stage progression and AD development. To date, we have succeeded to identify 36,320 new microglial enhancers including 937 regions that become different with age, and the analysis of coding regions regulated by the enhancers using Hi-C technology is in progress. As enhancers are activated in a cell-type specific manner, one can expect the development of novel technology that specifically controls the age-related functional alteration of microglia.

3) Mechanism of emergency myelopoiesis

Unlike steady-state hematopoiesis, hematopoiesis triggered at infection, irradiation and anti-cancer therapy is biased toward myeloid cell differentiation and production, that is "emergency myelopoiesis". However, due to the fluctuation of cell-surface marker Sca-1 on hematopoietic stem progenitor cells (HSPCs), it has long been difficult to understand bona-fide emergency myelopoiesis. Recently, our laboratory succeeded in identifying a novel HSPC marker CD86 with less fluctuation during emergency myelopoiesis (*Blood* 2020) (**Fig. 1**). Using this unique marker, we will elucidate the bona fide mechanism of emergency myelopoiesis.
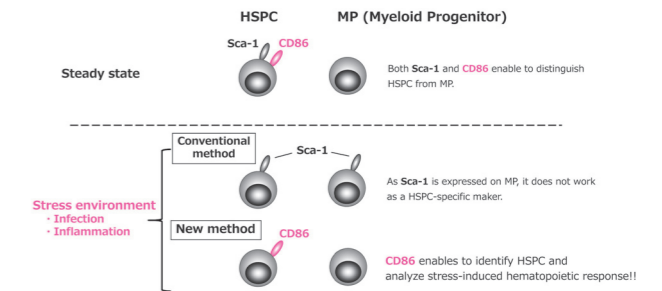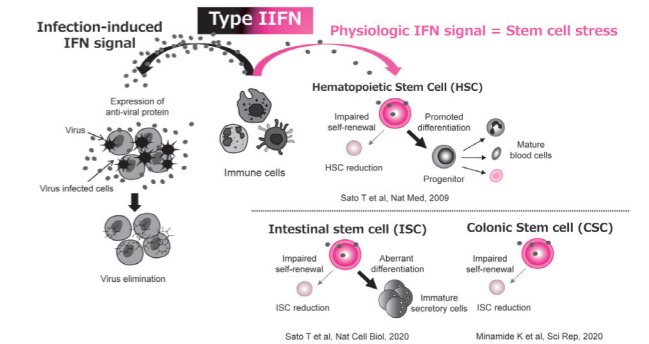
2. Research on tissue stem cells

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

Even under the steady-state, type I interferons (IFNs) are consistently produced, albeit in trace amounts, so called "physiologic type I IFNs". We previously reported that the physiologic 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 achievement, we examined the significance of physiologic type I IFNs in intestinal stem cells (ISCs) and found that it reduces the number and function of ISCs, resulting in the promoted differentiation into secretory progenitors (*Nat Cell Biol* 2020) (**Fig. 2**). Similarly, physiologic type I IFNs impaired the stemness of colonic stem cells (CSCs), leading to the defective colonic regeneration with lethality in a DSS colitis model (*Sci Rep* 2020) (**Fig. 2**).

Although several types of cells are synchronously involved in the damage-induced epithelial regeneration, it remains unclear to what degree each population contributes to the overall epithelial regeneration. Using a combination of genetic lineage tracing, single-cell gene expression profiling, and organoid-formation assays, we characterized the heterogeneity of epithelial stem cells in the radiation-damaged intestine. As a result, we found that the main cell of origin after intestinal injury originated from Lgr5$^+$ cells (*Sci Rep* 2020).

2) Establishment of human tongue cancer organoid biobank

Oral cancer has an increasing trend of 300,000 new cases per year worldwide. Two-thirds of them are tongue cancers (TCs), and in advanced cases, they become refractory to treatment and have a poor prognosis, and causal genes have not been identified. Under these backgrounds, we succeeded in establishing a human tongue cancer organoid culture system, which was also applicable to other types of oral cancers and esophageal cancer. The TC organoid lines can reproduce the original TC tissues and are useful for accurate risk grading and predicting prognosis at early stages of TCs. In addition, we identified new therapeutic target molecules (submitted). We aim to develop fundamental technologies that lead to personalized treatment.

Publications

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2. Asano J, Sato T, Ohteki T. Autophagy detection in intestinal stem cells. **Methods Mol Biol** 2171, 115-125 (2020). doi: 10.1007/978-1-0716-0747-3_7.
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5. Kanayama M, Izumi Y, Yamauchi Y, Kuroda S, Shin T, Ishikawa S, Sato T, Kajita M, Ohteki T. CD86-based analysis enables observation of bona fide hematopoietic responses. **Blood** 136, 1144-1154 (2020).

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. A novel pathogenesis of atrial fibrillation unraveled from Exon-Wide Association Study (ExWAS)

Genome-wide association studies (GWAS) have the purpose of predicting the risk of developing disease and identifying new disease pathways and new drug seeds. Nearly 20 years have passed since GWAS have been deployed, but there are few new disease pathways and new drug seeds identified from GWAS. This may be due in part to the low association (odds ratio) of single nucleotide polymorphisms (SNPs) found in GWAS to disease and the identification of most SNPs in non-gene regions. On the other hand, SNPs with high odds ratios are identified by exon-wide association analysis (ExWAS) and SNPs are exons, that is, on genes and thus ExWAS may overcome the above-mentioned problems of GWAS.

We identified SNPrs202011870 with an odds ratio of 3.617 in ExWAS in collaboration with RIKEN. In another Japanese cohort (3378 atrial fibrillation, 641 controls), rs202011870 was reproduced to be associated with the development of atrial fibrillation. The SNP resides on a cytoplasmic protein called Tks5 and replaces Lys at position 396 with Arg (L396R). Tks5 is abundantly expressed in macrophages and has been shown to be involved in podosome formation caused by protein kinase C (PKC) activation and associated migration and tissue infiltration of macrophages. Macrophages carrying Tks5 L396R were shown to promote podosome formation and migration / tissue infiltration even in a controlled state without PKC stimulation. This study successfully showed a new pathogenic mechanism of atrial fibrillation and drug discovery seeds from ExWAS.

2. Analysis of novel mechanism developing dilated cardiomyopathy (DCM) and atrial fibrillation (AF) in *Rbm20* mutant mice

RBM20, encoding RNA binding motif protein 20 (RBM20), was identified as one of the causative genes for DCM. RBM20 is a major regulator of heart-specific alternative splicing of the *TTN* gene, which is found to be most frequently mutated in patients with DCM. Extensive searches for mutations in *RBM20* in patients with DCM revealed a hotspot of missense mutations in a highly conserved RSRSP stretch, within an arginine/serine (RS)-rich region. Recently, the RSRSP stretch has been found to be critical for the nuclear localization of RBM20, and mutations in this stretch resulted in the loss of RBM20-dependent alternative splicing. Clinically, the DCM with an *RBM20* mutation were reported to show the more severe cardiac phenotypes including AF compared to the DCM caused by mutations in other genes. To understand the relationship between the mutation in RSRSP stretch and cardiac phenotypes, we generated *Rbm20^{S637A}* knock-in and *Rbm20* knock-out mice by CRISPR/Cas9-mediated genome editing. Although both *Rbm20^{S637A}* and *Rbm20* KO mice lost RBM20-dependent alternative splicing, only *Rbm20^{S637A}* mice showed severe cardiac phenotypes mimicking clinical patients (Sci Rep. 2020. 10: 17894.). A molecular feature of the *Rbm20^{S637A}* mutation is the presence of RBM20^{S637A} protein in the cytoplasm of cardiomyocytes. Considering that the *Rbm20^{S637A}* mutation specifically led to the DCM phenotype, it is reasonable to assume that cytoplasmic RBM20^{S637A} protein contributes to the development of DCM. Now, we are investigating the function of cytoplasmic RBM20^{S637A} protein in detail.

3. Understanding the molecular mechanisms of congenital heart diseases and cardiac cell specification during development

We are focusing on *Tbx5* gene, a member of the T-BOX family transcriptional factors, and the mutations of *TBX5* genes cause Holt-Oram syndrome with upper limb abnormalities and congenital heart defects (CHDs). To understand the molecular mechanisms of these diseases, we study the transcriptional network to specify heart cell fate during development (Morita & Takeuchi, *MMCHDPH* 2020). We found a novel surface marker, GPC5 as Tbx5 positive cells expressed in the left ventricle and the atria cardiomyocytes by transcriptome analysis. Interestingly, overexpression of GPC5 lead to progressive cell proliferation in the progenitor cells and tumorigenesis in human mesenchymal stem cells (Takeuchi et al., *PLOS ONE* in press 2020).

Highlight

One notable characteristic of the *Rbm20^{S637A}* mice is the development of AF (Fig.). It is generally very difficult to induce AF in mice, and spontaneous AF is very unusual. In previous studies, few transgenic strains have been reported to develop spontaneous AF. In contrast to previous AF mouse models, *Rbm20^{S637A}* mice is the first mouse strain to show spontaneous, persistent AF with only a single nucleotide substitution, mimicking a clinical patient. *Rbm20^{S637A}* mice offer a novel path for future therapeutics as a unique mouse model for not only DCM but also AF.
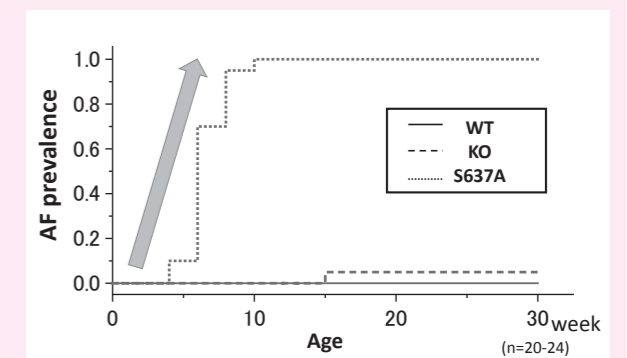

Fig. *Rbm20^{S637A}* mouse developed AF at a very high rate.

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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   Alapati AIMAITIJIANG, Wenyu LIU, Gerel MELIG, Tingting ZHANG, Ryota TSUKAHARA,
                      Ayumi ITABASHI, Akina KOSAKU, Mariko NAGANE
Research Student   Deng SHENGHUAN, Hirotaka YOSHIDA

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 2020 are mainly on two subjects: 1. Characterization of fetal hematopoiesis, and 2. Characterization of cancer stem cells and their niche.

Research Projects

1. Involvement of transcription factor Sox17-mediated GIMAP6 expression in the hematopoietic capacity in midgestation mouse dorsal aorta

In mouse ontogeny, hematopoietic stem cells (HSCs) first arise in intra-aortic hematopoietic clusters (IAHCs) of dorsal aorta in aorta-gonad-mesonephros (AGM) region at midgestation. In previous studies, we reported that introduction of a transcription factor Sox17, which is expressed in IAHCs, into CD45^{low}c-Kit^{high} cells, which are one component of IAHCs, maintains the hematopoietic ability and the formation of hematopoietic cell clusters in vitro. Moreover, we revealed that Sox17-induced Notch1 and adhesion molecules expression is important to maintain the undifferentiated state and the cluster formation. In the present study, we search a novel Sox17 downstream target involved in the maintenance of HSCs. First, we demonstrated that the expression of the genes encoding the GIMAP family, each member of which has GTPase activity, was high in CD45^{low}c-Kit^{high} and Sox17-expressing cells in E10.5 AGM regions by RNA-sequence analysis. Moreover, the expression level of the Gimap gene family was increased in Sox17-ERT-transduced cells by a tamoxifen-induced Sox17 nuclear translocation. Next, the number of CFU-Mix (multi-lineage) colonies was significantly increased in the Gimap6-transduced cells without Sox17 overexpression. Moreover, the number of CFU-Mix colonies was decreased by shRNA-mediated downregulation of Gimap6 in Sox17-transduced cells. We further examined the subcellular localization of GIMAP family using GIMAP-GFP fusion proteins. Interestingly, unlike other
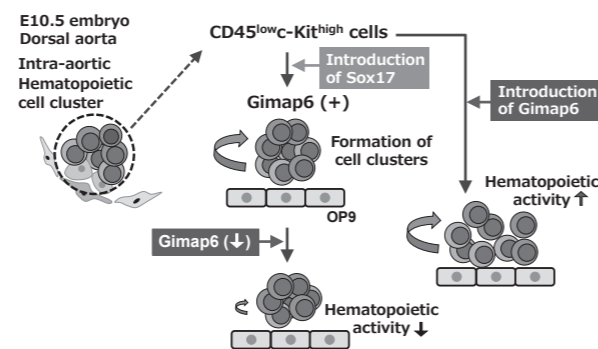

Figure 1: Model of the role of Sox17-induced Gimap6 expression in the hematopoietic ability of intra-aortic hematopoietic cell clusters

family members, GIMAP6 showed a dot-like distribution around the nucleus and a similar distribution of LC3, which has an important function in autophagosome formation. These results suggest that Sox17-induced Gimap6 expression contributes to the maintenance of the hematopoietic activity of IAHCs in midgestation mouse. (Figure 1).

2. Self-construction of glioma stem cell (GSC) niche by GSC-derived dead-cell particles

Gliomas are the most frequent primary brain tumors characterized by rapid and invasive growth into the brain parenchyma, which enables them to escape from surgical resection. The most malignant glioma, also known as glioblastoma multiforme, typically recurs within 1 year despite treatment with the anti-glioma drug Temozolomide (TMZ), and even combination therapy with the anti-angiogenic antibody Avastin has no effects on overall survival. Since it is such an intractable cancer with the poorest prognosis (5-year survival rate is <10%), the social demand for the development of new successful therapies is high.

"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. Spontaneous necrosis is a defining feature of glioblastomas (GBMs), the most malignant glioma. Despite its strong correlations with poor prognosis, how necrosis contributes to glioma progression remains unclear. By using propidium iodide staining and flow cytometry-based gating, we first isolated a necrotic particle spontaneously arising from glioma cells. This type of necrotic particles was subsequently characterized as "autoschizis-like products (ALPs)" according to their morphologies; loss of membrane integrity, organelle leakage, self-excisions of cytoplasm, intact nuclear envelope and karyolysis. Most of ALPs express the phosphatidylserine and were specifically engulfed by a minor subpopulation of CD204(+)CD11c(+) GM-CSF-primed Mϕs. Particularly, GSC-derived ALPs displayed the highest activity for the development of CD204(+) CD11c(+) Mϕs. Unexpectedly, Il12b gene encoding a major cytokine to induce Th1 antitumor immune response was identified as a specific marker for ALPs-educated Mϕs, but IL-12 protein evidently enhanced the sphere-forming ability of GBM patient-derived cells. Finally, higher expression of Il12b was closely associated with poor prognosis in recurrent GBM patients. These results highlight a self-expanding strategy of GSCs by modulating Mϕ phenotypes and TAM development through dead-cell particles, providing insights into the beneficial roles of necrosis in glioma malignancy.
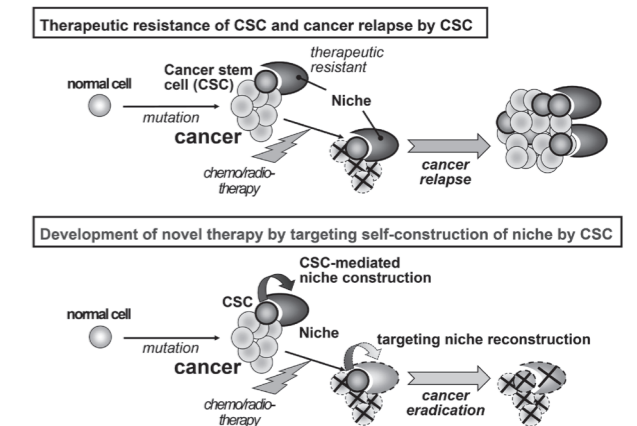

Figure 2: The concept of CSC-mediated niche construction and cancer eradication

Publications

[Original Article]
Takahashi S, Nobuhisa I, Saito K, Gerel G, Itabashi A, Harada K, Osawa M, Endo TA, Iwama A, Taga T. Sox17-mediated expression of adherent molecules is required for the maintenance of undifferentiated hematopoietic cluster formation in midgestation mouse embryos. Differentiation 115: 53-61, 2020

Tabu K, Liu W, Kosaku A, Terashima K, Murota Y, Aimaitijiang A, Nobuhisa I, Hide T, Taga T. Glioma stem cell (GSC)-derived autoschizis-like products confer GSC niche properties involving M1-like tumor-associated macrophages. Stem Cells, 38: 921-935, 2020

[Review Article]
Taga T and Tabu K. Glioma progression and recurrence involving maintenance and expansion strategies of glioma stem cells by organizing self-advantageous niche microenvironments. Inflammation and Regeneration, 40: 33, 2020

Anani M, Nobuhisa I, and Taga T. Sry-related high mobility group box 17 functions as a tumor suppressor by antagonizing the Wingless-related integration site pathway. Journal of Cancer Prevention, 25: 204-212, 2020

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. Molecular recognition mechanisms between T cell regulatory receptors and signal transduction molecules

T cells play an important role in the human immune system. In addition to antigen-specific activation signal via the T cell receptor (TCR) by binding to major histocompatibility complex (MHC), T cell activation is precisely regulated by non-antigen-specific signals via CD28 family receptors, such as CD28, cytotoxic T-lymphocyte antigen 4 (CTLA-4), and inducible T-cell co-stimulator (ICOS). The signals are transmitted by being recognized the intracellular consensus sequences of CD28 family receptors by the SH2 domains of the signaling molecules of Grb2, Gads, and PI3K p85. It will be useful to elucidate the detailed molecular mechanisms of the recognition between these receptors and signaling molecules based on the three-dimensional structures, for the development of novel drugs which prevent the autoimmune disease or the rejection of an organ transplant.

We have previously determined the crystal structures of the complex of the peptides containing the consensus sequence of CD28 or ICOS and the SH2s of signal transduction molecules of Grb2, Gads, and PI3K p85. However, the structure of the complex of the peptide derived from CTLA-4 and the SH2 was unclear. Recently, we have determined the crystal structure of the complex of the CTLA-4 peptides containing the consensus sequence and the C-terminal SH2 domain of PI3K p85, by X-ray crystallographic analysis with high accuracy of 1.1 Å resolution (Iiyama *et al.*, 2021). The obtained structure (Fig. 1)
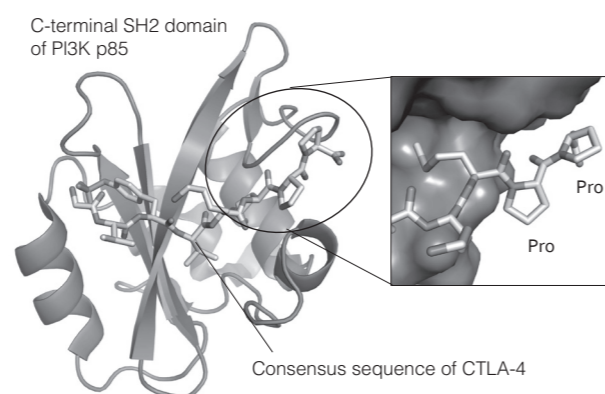

C-terminal SH2 domain of PI3K p85

Pro

Pro

Consensus sequence of CTLA-4

Fig.1 Crystal structure of the C-terminal SH2 domain (ribbon model) complexed with the consensus sequence of CTLA-4 (stick model).

reveals that two continuous proline (Pro) residues, which are characteristic of the sequence of CTLA-4 peptide, play an important role in the binding affinity to the SH2. By integrating with previously reported structural information of the complexes of CD28 family and the signal transduction molecules, rational design of small molecules that control the function of T cells is now underway.

This work is performed in collaboration with Professor Masayuki Oda at Kyoto Prefectural University.

2. Discovery of new drug seeds for Huntington's disease

Huntington's disease is an inherited disease that causes the progressive degeneration of nerve cells in the brain, in which mutant Huntingtin (Htt) proteins abnormally interacts with an essential DNA damage repair protein Ku70 in neurons and degenerate its function. In this study, we explored new drug seeds to interrupt the abnormal interactions between them. Finally, we succeeded in discovering three small chemicals with therapeutic effects. According to the dynamic light scattering analysis (Fig. 2), these chemicals most likely interact with mutant Htt and change its dynamics and aggregative propensity.

This work is performed in collaboration mainly with Professor Okazawa in our institute, and with several research groups at Tohoku University, Keio University, National Institute of Advanced Industrial Science and Technology, and Gladstone Institute.

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
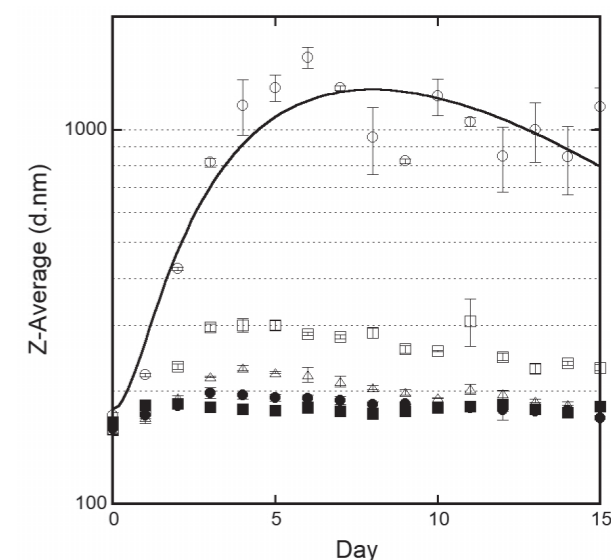

Fig.2 The three chemicals, 7H (filled circle), Ang III (filled square), and LH-RH fragment (filled triangle), affect the dynamic light scattering measurements of a mutant Htt aggregation (open circle) *in vitro*.

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

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Frontier Research Unit
Laboratory of Oxygen Biology

Associate Professor   Koh Nakayama, 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, in development, 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. Machinery of tumor growth mediated by pyruvate dehydrogenase PDH

Cancer cells often exhibit glycolytic metabolism in normoxia (also known as Warburg effect); however, the molecular machinery of how such metabolism is formed has not been clarified. We analyzed PDH and identified that PDH-E1β subunit is downregulated during chronic hypoxia. This downregulation is sustained upon reoxygenation, therefore inducing glycolytic metabolism in breast cancer cells even under normoxic condition. Furthermore, knockdown of PDH-E1β in breast cancer cells caused a Warburg effect-like metabolism, which also indicates the importance of PDH-E1β downregulation on inducing glycolytic metabolism in cancer cells. Importantly, PDH-E1β-KD cells formed smaller tumors than the control cells in nude mice, indicating that sustained glycolytic metabolism is not sufficient to promote tumor growth, and pointing to a possibility of cancer cells utilizing oxidative phosphorylation for their efficient energy production.

2. Role of nuclear PDH on histone acetylation and gene expression

Recently, PDH was shown to localize in the nucleus. We also demonstrated that PDH localizes in the nucleus
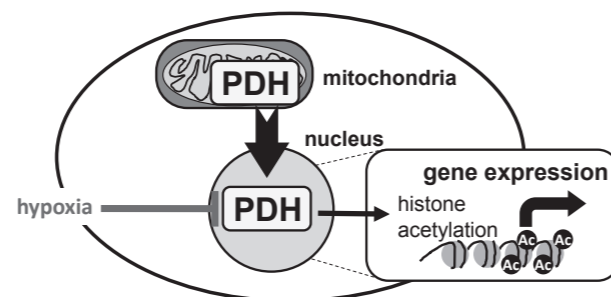

Figure Gene expression regulated by nuclear PDH
PDH exists both in mitochondria and nucleus. Nuclear PDH promotes histone acetylation and regulates gene expression. Nuclear PDH activity is inhibited under hypoxic conditions.

in breast cancer cell lines. PDH formed a large enzyme complex in the nucleus similar to that in the mitochondria. When mitochondrial and nuclear PDH were compared under hypoxic condition, mitochondrial PDH was efficiently phosphorylated, whereas nuclear PDH was not. Alternatively, nuclear PDH was efficiently downregulated under hypoxic condition. PDH promotes histone acetylation, and knockdown of PDH reduced the level of acetylated histone H3. Similarly, prolonged hypoxic condition inactivated PDH, reduced histone H3 acetylation, and altered the expression of genes involved in cell death, immune response and hypoxic response in breast cancer cell lines (Figure). Gene expression and metabolism are critical factors involved in cancer progression. PDH is a key molecule regulating both factors, and we further try to understand the regulatory mechanism of PDH.

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 novel amyloid hypothesis which corrects the previous understanding that extracellular Amyloid-beta plaque is the most upstream.
- Discovery of YAP protein as a new gene therapeutic target for Alzheimer's disease

【Biochemical Pathophysiology】
- Elucidation of phosphoinositide molecular species-specific signal transduction mechanism

【Pathological Cell Biology】
- Discovery of Wipi3 as an essential molecule for alternative autophagy
- Elucidation of the important role of alternative autophagy in neuronal cell viability

【Developmental and Regenerative Biology】
- Discovery of the involvement of prostaglandin E2 in cell competition

【Stem Cell Biology】
- Elucidation of the mechanisms of skin aging

【Immunology】
- Elucidation of the mechanism that regulates B cell development through molecular interactions within glycocalyx.
- Discovery of a genetic rare variant associated with Guillain-Barré syndrome

Department of Neuropathology

Professor	Hitoshi Okazawa
Practical professor	Kazuhiko Tagawa
Project Lecturer/Part-time Lecturer	Haruhisa Inoue, Masaki Sone
Assistant professor	Kyota Fujita
Project Assistant professor	Hidenori Homma
Postdoctoral fellow (JSPS research fellow)	Hikari Tanaka

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. YAP-dependent necrosis occurs in early stages of Alzheimer's disease and regulates mouse model pathology

The principal pathological hallmark of neurodegenerative disease such as Alzheimer's disease is accumulation of abnormal protein in intracellular and/or extracellular space. In Alzheimer's disease, two major hallmarks are well-known; one is extracellular Amyloid-beta plaque, and the other is intracellular neurofibrillary tangle composed by tau protein. Although several therapeutic approaches have been tried, there are still not found any practical and successful candidates. In the current 15 years, clinical therapy using anti-amyloid beta antibody have been performed internationally. However, those therapies brought us the evidence of elimination of extracellular Amyloid plaque, but not recovery of their symptoms. These results indicate that it is already too late to start treatment after onset, and it is necessary to develop treatments for new molecular targets at the very early (phase 0) stage before extracellular amyloid aggregation.

Previously, we have identified several phosphorylated proteins such as MARCKS whose phosphorylation started prior to extracellular amyloid aggregation (Tagawa et al, Hum Mol Genet 2015). We also found that phosphorylated MARCKS (pSer46-MARCKS) was located in degenerative neurites along with the extracellular amyloid plaques (Fujita et al, Sci Rep 2016). The upstream signaling for Ser46-MARCKS phosphorylation was HMGB1-TLR4 signaling. We have succeeded to prevent the onset of Alzheimer's disease by administration of anti-HMGB1 antibody targeting to extracellular HMGB1 in the mouse model of AD (Fujita et al, Sci Rep 2016).

HMGB1 is a well-known molecule secreted from cells after necrosis (Scaffidi et al., Nature 2002). So, we have tried to measure the HMGB1 concentration in CSF, and interestingly, the level of HMGB1 was increased more in MCI patient CSF than in post-onset AD patients. This result indicates that the cell death has already occurred prior to clinical symptoms appearance. So, we have developed the novel technique to identify the active necrotic neurons using anti-pSer46-MARCKS antibody, and have found that the active necrosis has been evoked prior to cognitive impairment or extracellular amyloid-beta plaque development.

In addition, we have performed the detailed observations of human Alzheimer's disease-related neurons that have been created from genome edited human iPS cells by introducing Alzheimer's gene mutations. We have found that such necrosis is a new type of necrosis (TRIAD) because of the interaction between intracellular amyloid and YAP which is required neuronal survival.

Furthermore, we performed YAP supplementation by gene therapy on Alzheimer's disease model mice with the aim of normalizing the YAP dysfunction that causes necrosis. As a result, suppression of TRIAD necrosis, improvement of cognitive function, and suppression of extracellular amyloid accumulation were observed.

In this study, it was strongly suggested that the amyloid hypothesis, which considers extracellular amyloid aggregation to be the most upstream, should be corrected. Instead, 1) extracellular amyloid aggregation as a result of necrosis beginning with intracellular amyloid accumulation, 2) (a) necrosis was triggered by intracellular amyloid accumulation, and (b) secondary cell death has occurred in surrounding neurons, 3) Necrosis triggered by intracellular amyloid accumulation is TRIAD by dysfunction of YAP, and 4) it is possible to develop treatments such as gene therapy based on YAP function recovery in the future (Figure). In addition, 5) it was shown that the amount of CSF HMGB1 could be developed as a pre-onset molecular marker of Alzheimer's disease.

Highlight

The role of YAP in TRIAD

Among the multiple types of cell death, apoptosis and necrosis are typical types. Apoptosis, programmed cell death, are executed by activation of certain signal pathway by intracellular or extracellular events. On the other hand, necrosis occurs by vigorous stress (heat, radiation, hemorrhage, chemicals, etc.) and it was thought as passive style of cell death. From the pathological viewpoint, apoptosis shows the dominant feature like nuclear structural changes like chromatin condensation or nuclear membrane divergence, and cells get shrunk. Necrosis leads to cytoplasmic abnormality rather than nuclear abnormality, such as organelles swelling and rupture. Necroptosis are one of the necrosis, however, it is induced by certain signal pathway activation which are regulated by RIP1/3. TRIAD is atypical cell death, which requires inactivation of YAP-Hippo-pathway-related transcription, leading to the malfunction of factors for cellular survival (Hoshino et al., JCB 2006; Mao et al., Hum Mol Genet 2016; Mao et al., Cell Death Dis 2016).

YAP acts as a transcriptional co-factor with transcriptional factor (TEAD), and it is implicated that Hippo pathway contributes tumorigenesis, development, and so on. YAP is the downstream protein which orchestrates the output cellular effects. Our group have reported the neuronal isoform of YAP (YAPdeltaC) in 2006 (Hoshino et al., JCB 2006). YAPdeltaC has three isoforms (int13, int25, int61) and contains WW domain (protein binding domain which has PPXY motif) like YAP. However, YAPdeltaC lacks transactivation domain for p73 at C terminal. YAPdeltaC as well as YAP play dominant-negative role and repress the TRIAD.
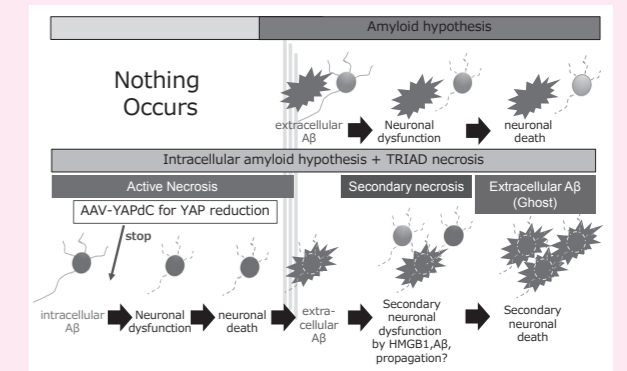
Publications

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Department of Biochemical Pathophysiology

Professor	Takehiko Sasaki, Ph.D.
Associate Professor	Junko Sasaki, Ph.D.
Assistant Professor	Junya Hasegawa, Ph.D.

Lipids are crucial for cell compartmentalization by membrane formation, energy storage, and signal transduction inside and outside cells. Our laboratory pays particular attention to a family of phospholipids called phosphoinositide (Fig. 1). Approximately 40 strains of gene-targeted mice lacking phosphoinositide kinase and phosphatase have been created and used for pathological studies of intractable diseases such as cancer, inflammatory diseases, and neurodegenerative diseases (Fig. 2). Also, by devising a new mass spectrometric technology for phosphoinositide measurement and applying it to pathological model mice and human disease samples, we are working to understand the mechanisms by which genetic abnormalities and environmental factors trigger pathological conditions. Besides phosphoinositides, we have recently identified novel lipids that fluctuate in cancers and inflammatory diseases. We are working on discovering their metabolizing enzymes, physiological functions, and molecular mechanisms of actions. With these research activities, we aim to deepen our understanding of the pathophysiological roles for phospholipids, present therapeutic targets for intractable diseases, and develop drug susceptibility prediction markers and disease stratification markers.

〈**Research Projects**〉

1. Developing predictive markers for cancer therapy by lipidomics

Phosphoinositide 3-kinases (PI3Ks) phosphorylate phosphatidylinositol 4,5-bisphosphate (PIP2) to produce phosphatidylinositol 3,4,5-trisphosphate (PIP3). Conversely, PTEN is the phosphatase that dephosphorylates the same position of the hydroxyl where PI3K phosphorylates, and converts PIP3 back to PIP2. These two enzymes or reactions play key roles in tumorigenesis and metastasis. High frequencies of gain-of-function mutations and amplification of *PIK3CA* encoding PI3K α as well as loss-of-function mutations and deletion of *PTEN* are found
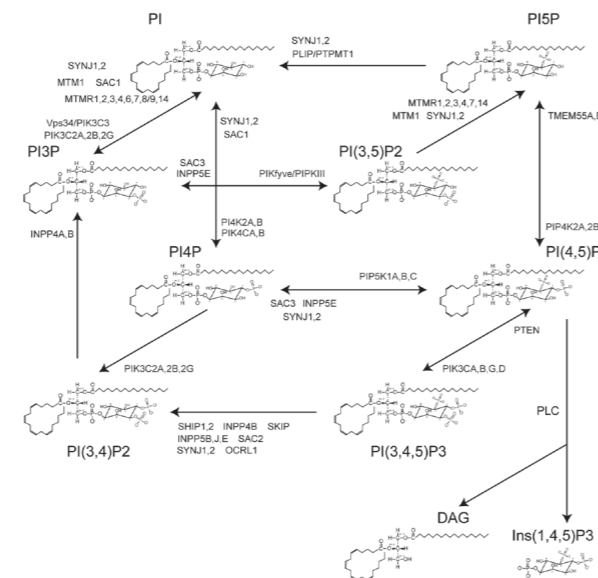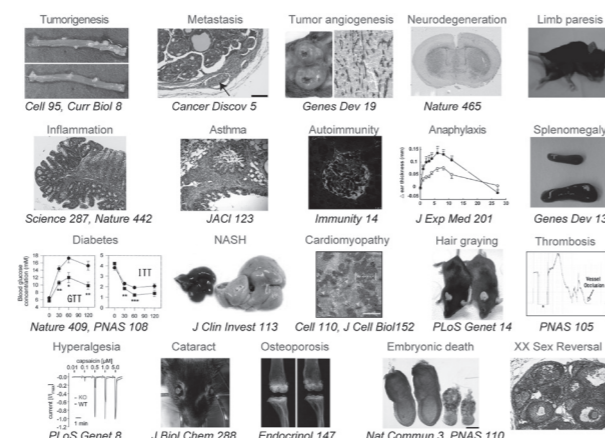

Fig.1

Fig.2

in various types of tumors. Given that PIP2 and PIP3 can regulate proteins involved in tumor cell proliferation, death, motility and invasion, PI3Ks are considered as potential therapeutic targets for cancers. A number of PI3K inhibitors have been developed and entered in clinical trials; however, they have so far had limited clinical success. Most studies have shown poor associations between drug responses and genetic alterations of *PI3KCA*, *PTEN* or other driver genes such as *RAS* and *HER2* encoding the upstream activators of PI3Ks. We have recently examined the fatty acyl profiles of phospho-

inositides and cell death responses to a series of anti-cancer agents in lymphoma cell lines. Multivariate analyses revealed significant association of the PIP2 profiles with susceptibility to PI3K inhibitors. Our results demonstrate that PIP2 acyl signatures would be useful for distinct stratification of lymphomas from the ordinary classification that predicts clinical benefits to PI3K inhibitors.

2. Identification of binding proteins specific for phosphoinositide species

In addition to the lymphomas described above, in tissues and cells obtained from human diseases and mouse models thereof, in many cases, the acyl group constitution is disordered in addition to the phosphorylation state of the head group. Therefore, we are searching for a target protein that specifically binds to phosphoinositide molecular species classified by an acyl group structure. Lipid bilayers containing species with identical headgroups were prepared, incubated with cell and tissue protein extracts, collected and analyzed by shotgun proteomics for identifying proteins that bind to each phosphoinositide species. Interestingly, there are many proteins having acyl group specificity while maintaining head group specificity. For some proteins, site-directed mutagenesis experiments identified amino acid residues involving acyl group recognition at the lipid bilayer interface, which was further confirmed by molecular dynamics simulations. From these results, we presume that a change in molecular species composition causes a massive twist in the output of signal transduction generated by membrane phosphoinositide and is involved in the formation of a disease state. The identification of pathologically relevant molecular species by disease lipidomics may shed light on the above-mentioned species-specific phosphoinositide binding proteins, and lysophospholipid acyltransferases that define the fatty acid composition, as new drug discovery targets.

3. Phosphoinositide metabolism to maintain femaleness

Mammalian sex is determined by the Y-linked Sry (Sex-determining region Y) gene. The supporting cell precursors of the fetal gonad differentiate into testicular Sertoli cells in the presence of Sry, while the cells differentiate into ovarian granulosa cells in the absence of Sry. These supporting cells commit to sexual differentiation of somatic and germ cells. Recent studies in mice have provided evidence that granulosa vs Sertoli cell fate decision is not necessarily permanent in postnatal life. In the adult ovary, Foxl2 (forkhead box L2) and estrogen receptors are required for maintaining granulosa cell fate by repressing male promoting signals, while in the adult testis, Dmrt1 (doublesex and mab-3 related transcription factor 1) and Sox9 (SRY-box 9) are required for maintaining Sertoli cell fate by repressing female promoting signals. Abolishing female promoting signals leads to loss of granulosa cell fate markers and up-regulation of Sertoli fate markers such as Sox9 and Dmrt1. We found that conditional knockout mouse mutants lacking phosphatidylinositol 3,4,5-trisphosphate (PIP$_3$) phosphatases exhibited female infertility. Histological examination of the mutant ovaries revealed that Sertoli-like cells emerged in the follicles. The female mutant mice had significantly higher levels of testosterone in the serum. Our results demonstrate that PIP$_3$ metabolism plays a key role in cell fate determination towards granulosa cells in the ovaries, and propose a possible etiology of DSD, disorders of sex development.

Publications

1. Nishio M, To Y, Maehama T, Aono Y, Otani J, Hikasa H, Kitagawa A, Mimori K, Sasaki T, Nishina H, Toyokuni S, Lydon J, Nakao K, Mak T, Kiyono T, Katabuchi H, Tshiro H, Suzuki A. Endogenous YAP1 activation drives immediate onset of cervical carcinoma in situ in mice. Cancer Sci. 111(10):3576-3587, 2020

Department of Pathological Cell Biology

Professor　　　　　　　　　　　　　　　Shigeomi SHIMIZU
Junior Associate Professor　　　　　　Satoko ARAKAWA
Project Junior Associate Professor　　Masatsune TSUJIOKA, Satoru TORII, Shinya HONDA
Assistant professor　　　　　　　　　　Hirofumi YAMAGUCHI
Project Assistant Professor　　　　　　Michiko MUROHASHI, Hajime SAKURAI
　　　　　　　　　　　　　　　　　　　Min Kyong SHIN, Hazuki ENDO, Saori NOGUCHI

This laboratory mainly focuses on understanding the molecular mechanisms of autophagy, programmed cell death, and organelle biology. 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 is considered to be essential molecules for the induction of autophagy. However, we found that cells lacking Atg5 can still form autophagosomes/autolysosomes and perform autophagic protein degradation when subjected to certain types of stress. Unlike canonical 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-dependent canonical pathway and an Atg5-independent alternative pathway. Ulk1 is an essential initiator not only for canonical autophagy but also for alternative autophagy. However, the mechanism as to how Ulk1 differentially regulates both types of autophagy has remained unclear.

In this year, we identified Wipi3 as a molecule essential for alternative autophagy, but which plays minor roles in canonical autophagy. Wipi3 binds to Golgi membranes and is required for the generation of isolation membranes. We establish neuron-specific Wipi3-deficient mice, which show behavioral defects, mainly as a result of cerebellar neuronal loss. The accumulation of iron and ceruloplasmin is also found in the neuronal cells. These abnormalities are suppressed by the expression of Dram1, which is another crucial molecule for alternative autophagy. Although Atg7-deficient mice show similar phenotypes to Wipi3-deficient mice, electron microscopic analysis shows that they have completely different subcellular morphologies, including the morphology of organelles. Furthermore, most Atg7/Wipi3 double-deficient mice are embryonic lethal, indicating that Wipi3 functions to maintain neuronal cells via mechanisms different from those of canonical autophagy. These results are epoch-making and will contribute greatly to the advancement of the research field of autophagy.

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. Apoptosis is carried out by the caspase activation and following substrates digestion. In this year, we focused novel form cell death, namely alternative anoikis. Now, we are searching physiological roles of alternative anoikis.

3, Novel organellar biology

Organelles are small, specialized structures in cells, which play specific roles to regulate various cellular events. The recent rapid development of imaging techniques have clarified the details of organelle dynamics, demonstrating that (1) various functional regions are dynamically formed within organelles, (2) organelle functions are made possible by the comprehensible actions of these functional regions. In this year, we found cross-talk between mitochondria and Golgi apparatus. We also found that various mitochondrila functions are influenced by the Golgi-localized molecules.
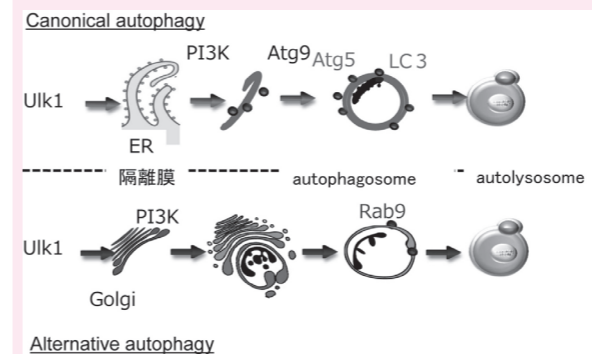
Figure1. Hypothetical model of autophagy.
There are at least two modes of autophagy, i.e. canonical and alternative autophagy. Canonical autophagy requires Atg5 and is originated from the ER membrane. In contrast, alternative autophagy occurs independently of Atg5 and is originated from the Golgi membrane.
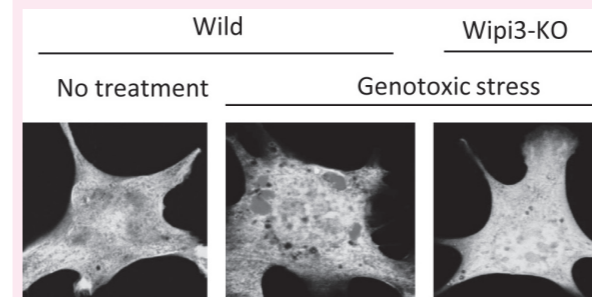

Figure2. Wipi3 is essential for etoposide-induced alternative autophagy
The mRFP-GFP tandem protein assay showed the essential role of Wipi3 in alternative autophagy. The indicated MEFs expressing a mRFP-GFP protein were left untreated or were treated with etoposide (10 μM). Red puncta indicate autolysosomes.

Figure3. Neurological defects in neuron-specific *Wipi3-KO* mice
Abnormal motor performance in *Wipi3-KO* mice at 10-weeks. The limb-clasping reflex was observed. The footprint assay indicated a motor deficit.

Alternative autophagy is an Atg/Atg7-independent type of autophagy that contributes to various physiological events. We here identified Wipi3 as a molecule essential for alternative autophagy, but which plays minor roles in canonical autophagy. Wipi3 binds to Golgi membranes and is required for the generation of isolation membranes. We established neuron-specific Wipi3-deficient mice, which showed behavioral defects, mainly as a result of cerebellar neuronal loss. The accumulation of iron and ceruloplasmin was also found in the neuronal cells. These abnormalities were suppressed by the expression of Dram1, which is another crucial molecule for alternative autophagy. Although Atg7-deficient mice showed similar phenotypes to Wipi3-deficient mice, electron microscopic analysis showed that they have completely different subcellular morphologies, including the morphology of organelles. Furthermore, most Atg7/Wipi3 double-deficient mice were embryonic lethal, indicating that Wipi3 functions to maintain neuronal cells via mechanisms different from those of canonical autophagy.

List of Publications

[Original paper]
1. Identification of a phosphorylation site on Ulk1 required for genotoxic stress-induced alternative autophagy. S. Torii, H. Yamaguchi, A. Nakanishi, S. Arakawa, S. Honda, K. Moriwaki, H. Nakano, S. Shimizu **Nature Commun 11**, Article number: 1754 (2020)
2. The ceramide analogue N-(1-hydroxy-3-morpholino-1-phenylpropan-2-yl)decanamide induces large lipid droplet accumulation and highlights the effect of LAMP-2 deficiency on lipid droplet degradation. Y. Kato, S. Arakawa, K. Terasawa, J.I. Inokuchi, T. Iwata, S. Shimizu, T. Watabe. **Bioorg. Med. Chem.** Lett. 30: 126891, 2020

3. ER-resident sensor PERK is essential for mitochondrial thermogenesis in brown adipose tissue. H. Kato, K. Okabe, M. Miyake, K. Hattori, T. Fukaya, K. Tanimoto, S. Beini, M. Mizuguchi, S. Torii, S. Arakawa, M. Ono, Y. Saito, T. Sugiyama, T. Funatsu, K. Sato, S. Shimizu, S. Oyadomari, H. Ichijo, H. Kadowaki, H. Nishitoh. **Life Science Alliance** 3: 3, e201900576, 2020
4. Sanguisorba officinalis L. derived from herbal medicine prevents intestinal inflammation by inducing autophagy in macrophages. A. Yasueda, H. Kayama, M. Murohashi, J. Nishimura, K. Wakame, K. Komatsu, T. Ogino, N. Miyoshi, H. Takahashi, M. Uemura, C. Matsuda, T. Kitagawa, K. Takeda, T. Ito, Y. Doki, H. Eguchi, S. Shimizu, T. Mizushima.

Scientific Reports 10, Article number: 9972 (2020)
5. Wipi3 is essential for alternative autophagy and its loss causes neurodegeneration. H. Yamaguchi, S. Honda, S. Torii, K. Shimizu, K. Katoh, K. Miyake, N. Miyake, N. Fujikake, H. Sakurai, S. Arakawa, S. Shimizu. **Nature Commun 11**, Article number: 5311 (2020)
6. Homeostatic p62 levels and inclusion body formation in CHCHD2 knockout mice. S. Sato, S. Noda, S. Torii, T. Amo, A. Ikeda, M. Funayama, J. Yamaguchi, T. Fukuda, H. Kondo, N. Tada, S. Arakawa, N. Watanabe, Y. Uchiyama, S. Shimizu, N. Hattori. **Human Molecular Genetics** in press 2021
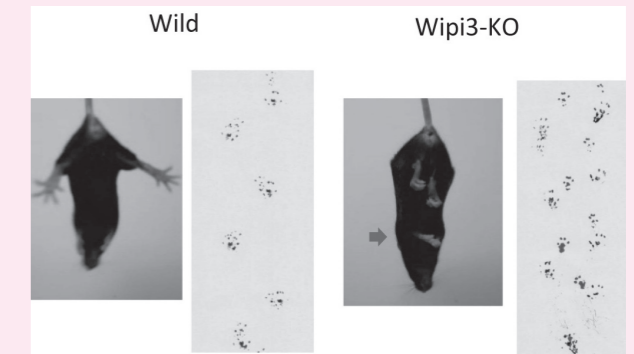
Department of Developmental and Regenerative Biology

Professor   Hiroshi Nishina, Ph.D.
Lecturer   Satoshi Kofuji, Ph.D.
Assistant Professor   Yasuhiro Nakano, Ph.D.

Our goal is to define the molecular mechanisms responsible for organ development, regeneration, and maintenance using mutant fish and knockout mice. To accomplish this goal, we have focused on defining signaling molecules and metabolic cues that regulate liver and brain formation and maintenance. Our studies will provide new insights into the precise molecular mechanisms that underlie organ failures found in human diseases and will lead to the development of new rational therapies for these disorders.

1. Research on early embryogenesis

Fertilized mammalian eggs repeatedly undergo cell division to generate the outer, middle, and inner germ layers that form the basis of organs. Through dynamic processes of cell migration and differentiation, the ectoderm arises from the upper layer of the blastoderm, and the mesoderm and endoderm form from the primitive streak. The primitive streak is therefore called the "first step towards cell differentiation" and is an extremely important tissue that initiates ontogeny. However, in the uterus of a pregnant mouse, the primitive streak is such a tiny tissue that it is difficult to analyze. Thus, there remain many questions about the molecular mechanisms driving the formation of the primitive streak. To address these questions, we have used mouse embryonic stem (ES) cells to generate a population of primitive streak-like cells. We have also established an experimental system to induce the differentiation of these cells into beating myocardial cells (derived from mesoderm), albumin-producing hepatocytes (derived from endoderm), and neurons that extend axons. Using this system, we have been successful in identifying various signaling molecules and metabolites required for primitive streak formation and differentiation.

2. Research on organogenesis

The individual sizes and shapes of living organisms are greatly influenced by earth's gravity. However, the mechanism by which organisms resist gravity to maintain these properties is largely unknown. Similarly, it is unclear why the organs of an individual organism perform their functions well only when they are properly sized and arranged in an orderly manner. To address these important issues, we have generated appropriate models by isolating gravity-sensitive medaka mutants and creating knockout mice. For example, using our gravity-sensitive medaka mutant, we unexpectedly discovered that the Hippo-YAP pathway plays an essential role in three-dimensional organogenesis. As a result of this information, we are currently analyzing the role of the Hippo-YAP pathway in mouse liver formation.

3. Research on organ homeostasis

Damage or senescence in cells can promote diseases such as cancer. Therefore, these abnormal cells need to be removed in order to maintain organ homeostasis. However, the mechanism by which these abnormal cells are eradicated is largely unknown. Using cultured cells derived from mouse liver or canine kidney, we have found that the Hippo-YAP pathway is involved in the elimination of abnormal cells. In a parallel project, we have shown that the MKK7-JNK pathway is essential for the constitutive functions of the mouse brain. We are analyzing the roles of these signaling pathways in maintaining the homeostasis of the mammalian liver and brain.

Highlight

Cell competition is a biological process by which unfit cells are eliminated from "cell society". We previously showed that cultured mammalian epithelial MDCK cells expressing constitutively active YAP were eliminated by apical extrusion when surrounded by "normal" MDCK cells. However, the molecular mechanism underlying the removal of active YAP-expressing cells was unknown. Over the past year, we have used high-throughput chemical compound screening to identify cyclooxygenase-2 (COX-2) as a key molecule triggering cell competition. Our work reveals that PGE_2 secreted in response to COX-2 activation engages its receptor EP2 on abnormal cells as well as on nearby normal cells. This engagement of EP2 triggers downstream signaling via an adenylyl cyclase-cyclic AMP-PKA pathway that, in the presence of active YAP, induces E-cadherin internalization leading to apical extrusion. Thus, COX-2-induced PGE_2 appears to be a warning signal to both abnormal and surrounding normal cells to drive cell competition.
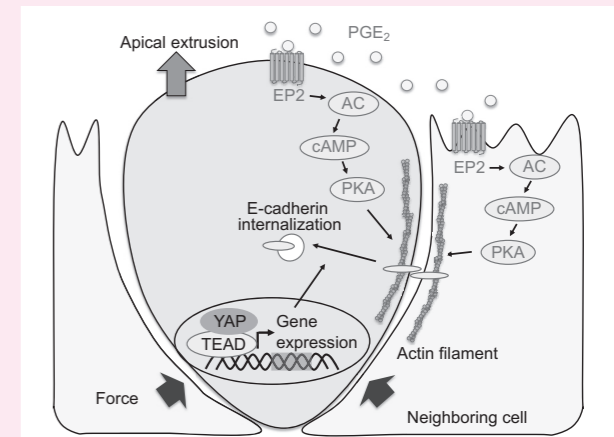
Fig.1.A Schematic model of molecular mechanisms for YAP-induced apical extrusion.

Publications

1. Erika Ishihara, Yuya Nagaoka, Toshiaki Okuno, Satoshi Kofuji, Mari Ishigami-Yuasa, Hiroyuki Kagechika, Kenya Kamimura, Shuji Terai, Takehiko Yokomizo, Yukihiko Sugimoto, Yasuyuki Fujita, Akira Suzuki and Hiroshi Nishina (2020) Prostaglandin E2 and its receptor EP2 trigger signaling that contributes to YAP-mediated cell competition. *Genes to Cells* 25, 197-214.

2. Hirofumi Omori, Miki Nishio, Muneyuki Masuda, Yosuke Miyachi, Fumihito Ueda, Takafumi Nakano, Kuniaki Sato, Koshi Mimori, Kenichi Taguchi, Hiroki Hikasa, Hiroshi Nishina, Hironori Tashiro, Toru Kiyono, Tak Wah Mak, Kazuwa Nakao, Takashi Nakagawa, Tomohiko Maehama and Akira Suzuki (2020) YAP1 is a Potent Driver of the Onset and Progression of Oral Squamous Cell Carcinoma. *Science Advance* 6: eaay3324

3. Kenya Kamimura, Takeshi Yokoo, Hiroyuki Abe, Norihiro Sakai, Takuro Nagoya, Yuji Kobayashi, Masato Ohtsuka, Hiromi Miura, Akira Sakamaki, Hiroteru Kamimura, Norio Miyamura, Hiroshi Nishina, Shuji Terai (2020) Effect of Diphtheria Toxin-Based Gene Therapy for Hepatocellular. *Cancers* 12(2), 472.

4. Miki Nishio, Yoko To, Tomohiko Maehama, Yukari Aono, Junji Otani, Hiroki Hikasa, Akihiro Kitagawa, Koshi Mimori, Takehiko Sasaki, Hiroshi Nishina, Shinya Toyokuni, John P. Lydon, Nishina, Kazuwa Nakao, Tak Wah Mak, Tohru Kiyono, Hidetaka Katabuchi, Hironori and Akira Suzuki (2020) Endogenous YAP1 activation Drives Immediate Onset of Cervical Carcinoma In Situ in Mice. *Cancer Science* 111, 3576-3587.

5. Takanobu Shimizu, Takeshi Nakamura, Hironori Inaba, Hiroaki Iwasa, Junichi Maruyama, Kyoko Arimoto-Matsuzaki, Takao Nakata, Hiroshi Nishina, Yutaka Hata (2020) The RAS-interacting chaperone UNC119 drives the RASSF6-MDM2-p53 axis and antagonizes RAS-mediated malignant transformation. *J. Biol. Chem.* 295, 11214-11230.

6. Nozomi Hanzawa, Koshi Hashimoto, Xunmei Yuan, Kenichi Kawahori, Kazutaka Tsujimoto, Miho Hamaguchi, Toshiya Tanaka, Yuya Nagaoka, Hiroshi Nishina, Sumiyo Morita, Izuho Hatada, Tetsuya Yamada, and Yoshihiro Ogawa (2020) Targeted DNA demethylation of the *Fgf21* promoter by CRISPR/dCas9-mediated epigenome editing. *Scientific Reports* 10, 5181.

7. Manami Kodaka, Kyoko Arimoto-Matsuzaki, Xiaoyin Xu, Zeyu Yang, Fengju Mao, Yue Guo, Junichi Maruyama, Kentaro Nakagawa, Kana Ishii, Chihiro Akazawa, Takuya Oyaizu, Naoki Yamamoto, Mitsuhiro Enomoto, Mari Ishigami-Yuasa, Nozomi Tsuemoto, Shigeru Ito, Hiroyuki Kagechika, Hiroshi Nishina, Yutaka Hata (2020) Characterization of a novel compound that promotes myogenesis via Akt and transcriptional co-activator with PDZ-binding motif (TAZ) in mouse C2C12 cells. *PLoS ONE* 15(4) e0231265.

8. Satoshi Kofuji and Atsuo T Sasaki (2020) [review] GTP Metabolic Reprogramming by IMPDH2: Unlocking Cancer Cells' Fueling Mechanism. *J. Biochem* 168(4):319-328.

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 Professors	Yasuaki Mohri, Ph. D., Hironobu Morinaga, Ph. D., Kyosuke Asakawa, Ph. D.,Liu Nan, M.D., Ph. D. Mariko Shimokawa Ph. D.
Joint Researcher	Yuko Muroryama, , Ph. D.,

Stem cell systems play fundamental roles in sustaining tissue turnover and homeostasis. Our goal is to understand the mechanisms of tissue homeostasis driven by stem cell systems in mammals and to apply that knowledge to better understand the mechanisms underlying tissue/organ aging, cancer development and other relevant diseases associated with aging. We further aim to apply this knowledge to drug discovery, regenerative medicine 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 are mini-organs located in the skin that 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 in each hair cycle. We previously identified the source of those melanocytes, "melanocyte stem cells" (McSCs), which are located in the hair follicle bulge and supply mature melanocytes required for hair and skin pigmentation (Nishimura EK et al. Nature, 2002). Subsequently, we identified similar McSCs in sweat glands (Okamoto N et al. PCMR, 2014). Further, we recently succeeded in identifying epidermal stem cells with sufficient self-renewing potential by using genetic tracing of stem cell clones (Liu N et al. Nature, 2019). As we have succeeded in identifying cultured human keratinocyte stem cells by deep learning-based automated cell tracking (Nanba D et al. *in press*), the system will provides a platform to a reliable and noninvasive technology for their quality control in regenerative medicine.

2) Mechanisms of stem cell maintenance

The underlying mechanisms of stem cell maintenance are a fundamental issue in stem cell biology and medicine. We have revealed that the niche microenvironment plays a dominant role in the fate determination of McSCs (Nishimura EK et al. Nature, 2002). That finding prompted us to further study the mechanisms involved and led us to demonstrate that hair follicle stem cells (HFSCs), which reside in the hair follicle bulge, serve as a functional niche for the maintenance of McSCs (Nishimura EK et al. Cell Stem Cell, 2010) (Tanimura S et al. Cell Stem Cell,

2011). We identified transforming growth factor β (TGF-β) secreted from HFSCs as niche-derived factors that is essential for McSC maintenance. Also we identified *Mitf* that encodes master regulator of melanocyte development and its downstream *Bcl2* as critical for maintenance. Furthermore, the deficiency of those genes all leads to the progressive expression of hair graying phenotype. Therefore, we concluded that the incomplete maintenance of McSCs either by defective signaling from the niche or by intrinsic defects in stem cells, results in an insufficient supply of mature melanocytes expressing the progressive hair graying phenotype (Figure 1).
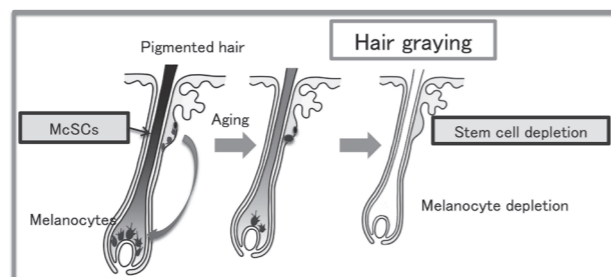

Figure 1: Aging and genomic stress abrogates the self-renewal of McSCs causing hair graying.

3) A self-renewal checkpoint underlies the quality maintenance of tissues

Physiological hair graying and hair thinning are typical outward signs of aging in mammals. We found that the incomplete maintenance of McSCs during the course of aging causes hair graying (Nishimura EK et al. Science, 2004). We then showed that genotoxic stress triggers/accelerates the aging process and abrogates the self-renewal of McSCs by triggering their differentiation (Inomata K et al. Cell, 2009). Further study of aged wild-type mice and progeroid mouse models, including *ATM*-deficient mice, revealed that a "self-renewal checkpoint",

which determines whether stem cells are qualified to self-renew or rather are forced to differentiate, maintains the quality of the stem cell pool and eliminates stressed/damaged stem cells from tissues (Inomata K et al. Cell, 2009). Similar checkpoint mechanisms have been found in HFSCs (Matsumura H et al. Science, 2016) and in epidermal stem cells (Liu N et al. Nature, 2019) by us and also in other somatic stem cells by other groups.

4) Dynamic elimination of aged stem cells causes hair follicle aging

To study the fate and dynamics of aged somatic stem cells, we performed *in vivo* fate tracing analysis of HFSCs and demonstrated that the dynamic elimination of HFSCs through their epidermal differentiation causes the stepwise miniaturization of hair follicles and eventual hair loss in mice. The DNA damage response in HFSCs causes proteolysis of Type XVII Collagen (COL17A1/BP180), a critical molecule for HFSC maintenance, to trigger HFSC aging that is characterized by the loss of stemness signatures and epidermal differentiation. Aged HFSCs are thus cyclically eliminated from the skin through their epidermal differentiation-mediated shedding from the skin surface, thereby causing hair follicle miniaturization (Figure 2). 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,
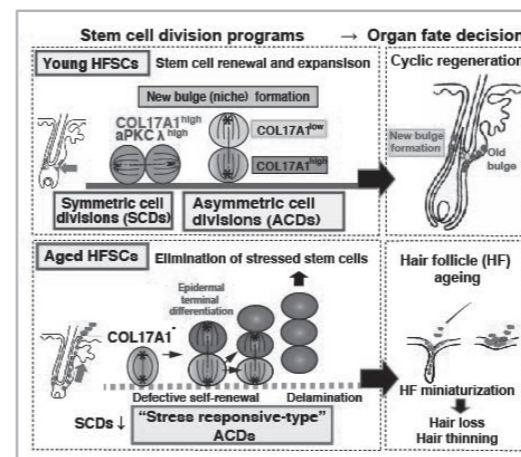

Figure 2: Schematic model of the distinct types of stem cell divisions that determine hair follicle regeneration and aging.

2016). Further, we analyzed the stem cell division axis in combination with fate tracing of HFSCs and identified the distinct stem cell division types that determine hair follicle regeneration and aging (Matsumura H et al. Science, 2016) (Figure 2).

5) Stem cell competition in the epidermis underlies skin homeostasis and aging

The skin protects living organisms from the outside world by acting as a barrier throughout the life-span, suggesting that the skin has more robust and flexible anti-aging mechanisms than mini-organs such as hair follicles. *In vivo* clonal analysis in mice revealed that the expression of COL17A1 fluctuates physiologically through genomic/oxidative stress-induced proteolysis, and that the resulting differential expression of COL17A1 in individual stem cells generates a driving force for cell competition (Figure 3). Clones that express high levels of COL17A1 divide symmetrically and outcompete/eliminate adjacent stressed clones that express low levels of COL17A1 and divide asymmetrically. Stem cells with higher potential or quality are thus selected for homeostasis, but their eventual loss of COL17A1 limits their competition, thereby causing aging. The resulting hemidesmosome fragility and stem cell delamination depletes adjacent melanocytes and fibroblasts to promote skin aging. Conversely, the forced maintenance of COL17A1 rescues skin organ aging, thereby indicating potential new approaches for anti-aging therapeutic intervention.
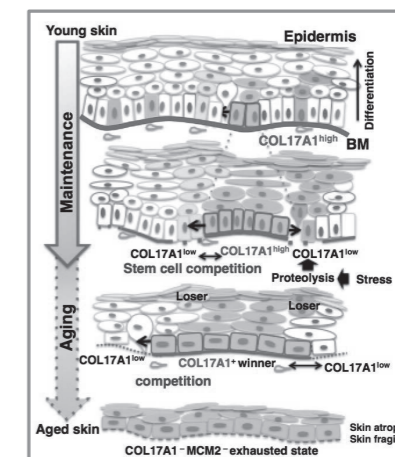
Figure 3: Stem cell competition orchestrates skin homeostasis and aging. SCD: symmetric cell division; ACD : asymmetric cell division.

Annual publications

Matsumura H, Liu N, Nanba D, Ichinose S, Takada A, Kurata S, Moringa H, Mohri Y, De Arcangelis A, Ohno S, Nishimura EK
Distinct types of stem cell divisions determine organ regeneration and aging in hair follicles.
Nature Aging, 1: 190-204, **2021**

Al-Busani H, Al-Sobaihi S, Nojima K, Tanemura A, Yaguchi T, Kawakami Y, Matsumura H, Nishimura EK, Yokozeki H, Namiki T
NUAK2 localization in normal skin and its expression in a variety of skin tumors with YAP.
J Dermatol Sci., 97(2): 143-151, **2020**

Invited lectures at international meetings
Emi K. Nishimura: Stem cell competition for skin homeostasis and aging : ISSCR 2020 Virtual, June 26-27, **2020**

Department of Immunology

Professor	Takeshi Tsubata, M.D., Ph.D.
Associate Professor	Takahiro Adachi, Ph.D.
Assistant Professor	Chizuru Akatsu, Ph.D.
Project Assistant Professor	Shuichi Kinpara, Ph.D.
Researcher	Feng Yang-yang, Ph.D., Alborzian Deh Sheikh, Amin, Ph.D.
Lecturer	Wang Ji-Yang, Ph.D.

Antibody responses to non-protein antigens such as polysaccharide and nucleic acids play crucial roles in host defense against pathogens, and autoimmune diseases. The mechanisms for antibody responses to non-protein antigens are distinct from those to protein antigens, but are largely unknown. We are elucidating mechanisms for antibody responses to non-protein antigens in normal immunity and autoimmunity, and also developing novel therapies and vaccines.

1. Study on the regulatory mechanisms of autoantibody production in systemic lupus erythematosus (SLE) and autoimmune neuropathy

Autoantibodies to nucleic acids and nucleic acid-related antigens are characteristically produced in patients with SLE, whereas patients with Guillain-Barré syndrome (GBS), an autoimmune neuropathy, produce autoantibodies to gangliosides, sialylated glycolipids, expressed in neuronal tissue. These autoantibodies are suggested to be pathogenic. Previously, we demonstrated that the inhibitory B cell co-receptor CD72 inhibits development of SLE (Xu et al. J. Immunol. 2013). We further showed that CD72 recognizes the RNA-related lupus self-antigen Sm/RNP, and inhibits antibody responses to Sm/RNP (Akatsu et al. JEM 2016). Although inhibition of production of anti-Sm/RNP antibody may play a role in prevention of SLE, patients with SLE produce autoantibodies to other self-antigens as well. We therefore analyzed binding of CD72 to various self-antigens using a self-antigen microarray in collaboration with Dr. Li at the University of Texas Southwestern. This analysis revealed additional lupus self-antigens recognized by CD72. We are currently addressing the role of this CD72 recognition in the regulation of SLE.

The inhibitory B cell-coreceptor Siglec-10 recognizes sialic acid as a ligand. By analyzing the sequence of Siglec-10 gene in patients with GBS, we showed evidence suggesting that Siglec-10 inhibits B cell response to gangliosides by recognizing gangliosides, thereby inhibiting development of GBS (Alborzian Deh Sheik et al. 2021).

2. Study on the B cell regulation by molecular interactions in glycocalyx

Glycans bound to lipids and proteins on the cell surface form a glycan-rich layer called glycocalyx. In the glycocalyx, glycan-mediated molecular interactions are thought to regulate cellular functions. However, little is known about the details. CD22 is an inhibitory co-receptor expressed in B cells and a lectin recognizing sialic acid. CD22 forms a homotypic cluster by recognizing sialic acids on neighboring CD22, and also associates with BCR in a manner dependent on sialic acid. We recently showed that these sialic acid-dependent interactions are crucial in normal B cell development and function (Akatsu et al. under revision, Alborzian Deh Sheik under revision). We also demonstrated in collaboration with Prof. Butcher at the Stanford University that CD22 regulates cell surface expression of the integrin $\alpha 4 \beta 7$ involved in lymphocyte homing to gut-associated lymphoid tissue through sialic acid-mediated interaction with $\alpha 4 \beta 7$ (Ballet et al. Nat Immunol. 2021).

3. Study on the mechanisms for antibody production to polysaccharides

Antibody production to polysaccharides plays a crucial role in infection immunity. This antibody response is independent on T cell help, but the mechanism is largely unknown. We are currently working on elucidating the mechanisms how B cells respond to polysaccharide in the absence of T cell help to produce specific antibodies.

4. Development of novel therapies for autoimmune diseases and vaccines.

We are developing vaccines that efficiently induce antibody production to the target molecules by conjugating a part of the target molecules to a highly immunogenic carrier molecule, and applying this vaccine technology to the development of cancer vaccines and vaccines against COVID-19. We are also developing a new therapy for autoimmune diseases that expands regulatory B cells, because regulatory B cells are suggested to suppress development of some autoimmune diseases.

Highlight

Identification of a genetic variant associated with Guillain-Barré syndrome (GBS), an autoimmune neuropathy

Guillain-Barré syndrome is an autoimmune neuropathy associated with production of autoantibodies to gangliosides, sialylated glycolipids rich in neural tissues. Anti-ganglioside autoantibodies are suggested to play a role in development of GBS. Although evidence suggests that GBS involves genetic factors, little is known about the details. Siglec-10 is an inhibitory B cell co-receptor that recognizes sialic acid as a ligand. In collaboration with Prof. Kusunoki at Kidai University, we analyzed sequence of the Siglec-10 gene in patients with GBS, and found that the Siglec-10 variant in which the arginine at the position 47 is replaced by glutamine (R47Q) in the ligand-binding domain is significantly accumulated in patients with GBS. We further demonstrated that the R47Q mutant fails to recognize gangliosides whereas wild-type Siglec-10 binds to various gangliosides (Alborzian Deh Sheik et al. 2021). These results suggest that the Siglec-10 R47Q is involved in development of GBS. When B cells recognize gangliosides on the surface of neurons as antigens, wild-type Siglec-10 may inhibit B cell activation by recognizing gangliosides (Fig.). In contrast, the Siglec-10 R47Q mutant fails to inhibit activation of anti-ganglioside B cells due to failure in recognizing gangliosides, thereby allowing development of GBS. Siglec-10 R47Q is a rare variant as the allele frequency in Japanese is around 1%. In general, rare variants have bigger impacts on disease development than common variants. Therefore, Siglec-10 R47Q is likely to play an important role in development of GBS. Rare variants often show regional distribution. Indeed, Siglec-10 R47Q was so far found only in Japan and Southern China. Because GBS development is often triggered by infection especially Campylobacter jejuni infection, individuals carrying Siglec-10 R47Q may be able to reduce the risk of GBS by avoiding behavior causing Campylobacter jejuni infection.
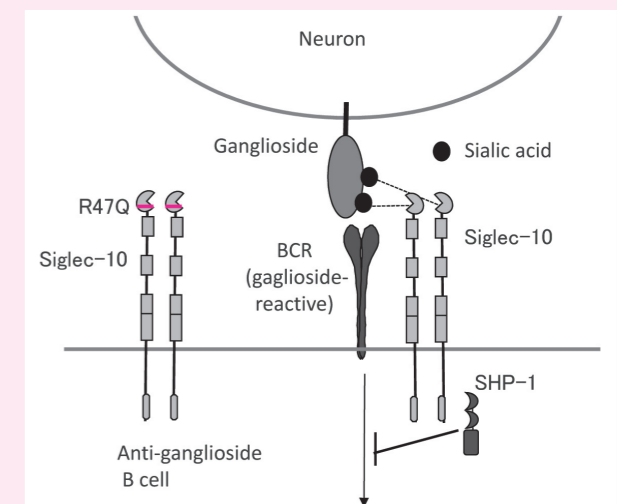

Fig. Siglec-10 suppresses production of autoantibodies to gangliosides
When anti-ganglioside antibody-producing B cells recognize gangliosides on the surface of neurons through B cell receptor (BCR), wild-type Siglec-10 that recognizes gangliosides inhibits B cell activation by activating tyrosine phosphatase SHP-1, thereby inhibiting production of anti-ganglioside autoantibodies. In contrast, Siglec-10 R47Q fails to suppress production of anti-ganglioside antibodies because Signelc-10 R47 is not able to recognize gangliosides.

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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 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 the genomic, epigenomic and proteomic changes underlying 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 is to understand the molecular mechanisms and establish practically useful modalities for the diagnosis and therapy for cancer and genetic diseases including intellectual disability and/or intellectual disability (ID/MCA).

1. We identified *miR-1293* as a miR for the development of miRNA-based cancer therapeutics. miR-1293 concurrently targets BRD4 and several DNA repair genes (APEX1, POLD4 and RPA1), inducing DNA damage and apoptosis in cancer cells.
2. We developed miR-634 ointment for improving EGFR TKI-based therapy for a cutaneous squamous cell carcinoma (cSCC).
3. We identified pitavastatin as a candidate of novel anticancer drug through a functional cell-based screening of an FDA-approved drug library. The combination of pitavastatin and capmatinib, a MET-specific inhibitor, dramatically reduced tumor growth.
4. We identified a rare missense variant in *OTUD5* in a boy with severe developmental delay and multiple congenital anomalies (MCA). *OTUD* is a responsible gene for a novel ID/MCA disorder, named LINKage-specific deubiquitylation deficiency-induced Embryonic Defects (LINKED) syndrome.

【Molecular Genetics】

We analyzed the functions of breast cancer-related molecules including BRCAs, and based on the results obtained, we aimed to elucidate the mechanism of breast oncogenesis and develop new therapeutic methods.

1. We searched for novel binding molecules of the breast cancer-related genes, BRCA1, BRCA2, and analyzed their functions and DNA damage repair mechanism in detail. In addition, we sought novel synthetic lethal interactions between BRCA1/2 and chemical compounds provided by Chemical Biology Screening Center at TMDU.
2. Focusing on the breast cancer invasion, we utilized a ductal model created by in vitro 3D culture to analyze the mechanism for disruption of the breast duct structure and basement membrane by estrogen.
3. Hereditary breast and ovarian cancer (HBOC) has a reduced function of DNA homologous recombination (HR) repair and shows a highly sensitive response (synthetic lethality) to DNA damaging agents such as PARP inhibitors. However, various types of acquired chemoresistance mechanisms were reported. We screened chemical compounds to explore novel factors that enhance the sensitivity of cancer cells in combination with a PARP inhibitor.

【Molecular Epidemiology】

1. We found in the consecutive autopsy cases of the JG-SNP database, heterozygotes of Werner syndrome mutation WRN pR369X (MAF=0.11%) can expect average length of life, albeit men carriers may prone to multiple primary cancer risk including lung cancer.
2. D-aminoacid oxidase (DAO) is an essential enzyme for H2S generation with cyto-protective effects. DAO pP103L, a hypomorphic allele and a risk factor for amyotrophic lateral sclerosis (ALS) was shown to be a risk factor for gastric cancer in men.
3. In addition to continuing the original birth cohort study in TMDU, we have prepared to initiate the collaboration with the ToMMo's Birth and Three-Generation Cohort and the Hamamatsu Birth Cohort groups, to further expand DOHaD research.
4. In collaboration with the Department of Otolaryngology, Keio University School of Medicine, we found that rs131702 of BCR gene is associated with severe tinnitus accompanied by a high degree of distress.

【Epigenetics】

1. We reported that *PEG11/RTL1* is responsible for the muscle- and brain-related defects in Kagami-Ogata and Temple syndromes, two genomic imprinting diseases occurred by paternal and maternal disomy of chromosome 14. Importantly, PEG11/RTL1 is expressed in the corpus callosum and corticospinal tract, therefore, it is likely that PEG11/RTL1 was deeply involved in brain evolution in eutherians.
2. We reported a new method for induce heart-like structure (heart organoid) from mouse ES cells. We also applied this protocol and succeeded to generate the heart organoid from human ES cells.
3. We have developed a new method for detecting proteins translated from human endogenous retrovirus (HERV)-derived genes. We proved its validity by detecting a protein from a HERV-derived gene conserved in Simiiformes.

【Medical Science Mathematics】

1. Participating in the International Cancer Genome Consortium (ICGC), we analyzed the whole genome of 2,658 cases across all cancer types for the effects of both coding and non-coding genomic regions on cancer. As a result, we found significant effects of mutations in long non-coding RNA and gene-regulatory regions. By cataloging and analyzing the functions of these genes, we have established a basis for new drug discovery for various types of cancer.
2. In order to elucidate how many cells from primary tumors could be contained in metastatic tumors, we developed a mathematical method to quantify the "founder cell population size" at the metastasis site using WES data of both the tumors. By applying our method to real data from colorectal cancer patients, we found metastases of populations of 3-17 cells. This phenomenon may have been cause of differences in treatment responses.

【Genomic Function and Diversity】

1. To dissect pathological mechanism of immunological diseases, we performed long-read RNA-seq of immune cell subsets and established their isoform catalogues. We also integrated genome-wide association studies (GWAS) and splicing QTL (sQTL) analyses to comprehensively identify sQTL genes involved in the diseases.
2. We performed eQTL analysis using synovial cells of rheumatoid arthritis patients to identify synovial cell-specific eQTL genes. By integrating the results with GWAS data, we identified eQTL genes involved in the disease pathogenesis.
3. In an international collaborative study, we performed GWAS meta-analysis of systemic lupus erythematosus in East Asians and revealed over a hundred of disease-susceptibility loci.
4. We established a pipeline to identify structural and repetitive sequence variants in the genome using sequence data obtained from long-read sequencers.

【Functional Genome Informatics】

1. We have developed a novel high-throughput RNA-seq system to obtain transcriptome data from phenotypic screening and cohort samples.
2. We have developed a machine learning model to generate gene expression matrices by deep learning of high-throughput RNA-seq and metadata.
3. We have published two papers on the analysis of disease mechanisms using the single-cell RNA-seq method developed in our lab: the first on the pathogenesis of psychiatric disorders using iPS cells from human diseases (Sawada T., Mol. Psychiatry. 2020); and the second on the quick response mechanisms of immune cells (Michida H., Cell Rep. 2020);

Department of Molecular Cytogenetics

Professor	Johji Inazawa, M.D., Ph.D.
Associate Professor	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 and to establish practical and useful modalities for the diagnosis and therapy of personalized medicine for cancer and genetic diseases, with the purpose of addressing unmet medical needs.

I. Development of microRNA-based cancer therapeutics

1) Development of anticancer drugs using tumor-suppressive miRNA.

Human microRNA (*miR*), a functional RNA consisting of approximately 22 bases, negatively controls gene expression by directly binding to the transcripts of the target gene. More than 2,500 *miRs* are known to exist in humans. Tumor-suppressive (TS)-*miR* therapy has attracted attention as a form of nucleic acid-based drug therapy. However, for this therapeutic strategy to become clinically applicable, the development of an efficient drug delivery system (DDS) capable of delivering TS-*miRs* into the targeted tumor cells is urgently required. We have previously identified that *miR-634*, a TS-*miR* which simultaneously and directly suppresses multiple genes related to cancer metabolism and cell survival, has been expected to be useful as a drug seed for the nucleic acid therapeutics. Indeed, we have demonstrated the therapeutic potential of LNP (lipid nanoparticles)-mediated delivery of *miR-634* for cancer therapy.

Here, we found that topical application of an ointment containing *miR-634* inhibited *in vivo* tumor growth without toxicity in a cutaneous squamous cell carcinoma (cSCC) xenograft mouse model and a DMBA/TPA-induced papilloma mouse model. Functional validation revealed that *miR-634* overexpression reduced glutaminolysis by directly targeting *ASCT2*, a glutamine transporter. Furthermore, enforced expression of *miR-634* synergistically enhanced EGFR tyrosine kinase inhibitors (TKIs)-induced cytotoxicity by triggering severe energetic stress *in vitro* and *in vivo*. Thus, we propose that topical treatment with *miR-634* ointment is a useful strategy for improving EGFR TKI-based therapy for cSCC (Inoue J et al. Mol Ther Oncolytics. 2020).

2) Exploration of novel *TS-miRs*

Nucleic acid therapeutics, including miRNA formulations, are now developed as a next-generation cancer treatment. To investigate novel *TS-miRs* for the development of miRNA-based cancer therapeutics, we screened a total of 2,565 *miRs*, which covered ~96% of the registered human *miRs*, on the basis of their growth-inhibitory effects in cancer cells. We identified *miR-1293* as a novel *TS-miR* targeting BRD4, a member of the bromodomain and extra-terminal domain (BET) protein family. Moreover, *miR-1293* directly suppressed several DNA repair genes (*APEX1*, *POLD4*, *RPA1*) by binding to its 3'UTR, inducing DNA damage and apoptosis in cancer cells. Concurrent suppression of BRD4 and these DNA repair genes synergistically inhibited tumor cell growth *in vitro*. *In vivo* therapeutic model showed that local administration of *miR-1293* suppressed tumor cell growth without any obvious adverse consequences. These results indicated that *miR-1293* might be a potent candidate for the development of miRNA-based cancer therapeutics (Takagawa Y et al. Mol Ther. 2020).

II. Establishment of novel therapeutic strategies based on precision cancer medicine

Exploring novel anticancer drugs based on drug repurposing (DR)

DR is a widely used strategy that seeks to identify new medical indications for drugs that are already approved for the treatment of an original disease(s). To find novel anticancer drugs for oral and esophageal squamous cell carcinomas (OSCC, ESCC) treatment, we performed a functional cell-based screening with HOC313-LM, an OSCC cell line, using an FDA-approved drug library containing 766 drugs. As a result, we identified pitavastatin as an anticancer drug candidate. One of the mechanisms by which pitavastatin inhibits cell growth might be the suppression of MET signaling through immature MET due to dysfunction of the Golgi apparatus. Moreover, the combination of pitavastatin with capmatinib, a MET-specific inhibitor, dramatically reduced tumor growth in *in vivo* experiments, suggesting that this combination is a useful therapeutic strategy in OSCC and ESCC (Xu B et al. Mol Cancer Res. 2020).

III. Molecular investigation of congenital disorders

Intellectual disability (ID) affects 2-3% of the population, often associated with multiple congenital anomalies (MCA). Due to the extensive genetic heterogeneity, the diagnosis is challenging and remains unknown for a large subset of cases. Since 2005, we have investigated 645 subjects with undiagnosed ID/MCA recruited from 23 institutes in Japan. First, we screened copy number variants (CNVs) by microarrays and detected pathogenic CNVs in 155 cases (24%). Next, 105 cases previously negative for pathogenic CNVs were screened for single nucleotide variants (SNVs) using a 75-gene custom panel. In total, pathogenic SNVs were identified in 20 cases (19%). Subsequently, aiming at detecting novel genes responsible for ID/MCA, six cases underwent trio whole-exome sequencing, of which novel ID/MCA genes were identified in two cases.

One of the novel genes is *OTUD5*, an X-linked gene encoding a K48/K63 linkage-specific deubiquitinating enzyme. We identified a rare *OTUD5* missense variant (p.Arg274Trp) in a male patient with severe developmental delay and multiple congenital malformations. The variant was inherited from the unaffected mother that had an extremely skewed X-inactivation pattern in peripheral blood DNA. We joined an international collaboration led by a group in the U.S. National Institutes of Health, in which a total of 10 male cases with *OTUD5* mutations were assembled for functional investigation. In vitro analyses showed that p.Arg274Trp caused partial mislocalization of OTUD5 to the cytoplasm and impaired deubiquitinating activity. This investigation demonstrated that hypomorphic *OTUD5* mutations are responsible for a novel ID/MCA disorder named LINKage-specific deubiquitylation deficiency-induced Embryonic Defects (LINKED) syndrome (Beck DB et al. Sci Adv. 2021).

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Department of Molecular Genetics

Professor   Yoshio Miki, MD. Ph.D.
Associate Professor   Akira Nakanishi, Ph.D.
Assistant Professor   Shigeaki Sunada Ph.D.
Project Assistant Professor Ji Shuting Ph.D.

BRCA1 and BRCA2 function for DNA double-strand break repair. While this function is a guardian to prevent carcinogenesis, it repairs DNA damage caused by anticancer drugs in cancer tissues, reduces cell death induction, and results on resistance to treatment. In addition, BRCA1, 2 have many other functions to maintain DNA stability, and their dysfunctions also induce breast carcinogenesis. We are trying to elucidate the mechanism of maintaining DNA stability by BRCA1, 2 and to develop a new breast cancer treatment method targeting DNA damage repair. Furthermore, since DNA damages induce PD-L1 expression, we studied the intracellular dynamics of PD-L1 and found that PD-L1 translocates to the nucleus and induces the expression of immune response genes. Our goal is to reveal the functions of molecules associated with breast carcinogenesis and to develop novel treatments for breast cancer based on this information.

1. Elucidation of the regulation mechanism of BRCA2 protein levels in the cell cycle

BRCA2 is a mediator of genome maintenance during the S phase of the cell cycle, with important implications for replication stress. BRCA2 expression levels are regulated in a cell cycle-dependent manner. We previously demonstrated that the highest and lowest levels of BRCA2 expression were reached during the S phase and M phase, respectively; however, little is known about the regulation of BRCA2 levels. In this study, we aimed to determine the regulation of BRCA2 expression and indicate the physiological significance of BRCA2 degradation during the cell cycle. We found that BRCA2 expression decreased in response to ubiquitin and heat-shock protein 27 (HSP27). In addition, BRCA2 amino acids 2241–2580 were degraded upon inhibition of protein synthesis with cycloheximide. To determine whether the degradation was caused by the ubiquitin-proteasome system, we analyzed the combined effect of MG132 and cycloheximide. This combination resulted in the degradation of Ub-BRCA2. Investigation of ubiquitination modification sites within this region is currently underway.

2. Acetylation-dependent regulation of PD-L1 nuclear translocation dictates the efficacy of anti-PD-1 immunotherapy.

We report that PD-L1 translocates from the plasma membrane into the nucleus through interactions with components of the endocytosis and nucleocytoplasmic transport pathways, regulated by p300-mediated acetylation and HDAC2-dependent deacetylation of PD-L1. Moreover, PD-L1 deficiency leads to compromised expression of multiple immune-response-related genes. Genetically or pharmacologically modulating PD-L1 acetylation blocks its nuclear translocation, reprograms the expression of immune-response-related genes and, consequently, enhances the anti-tumor response to PD-1 blockade. Thus, our results reveal an acetylation-dependent regulation of PD-L1 nuclear localization that governs immune-response gene expression, and thereby advocate targeting PD-L1 translocation to enhance the efficacy of PD-1/PD-L1 blockade.

3. Development of novel synthetic lethal therapy for chemoresistant tumors

Hereditary breast and ovarian cancer (HBOC) by genetic defects in BRCA genes has dysfunctional DNA homologous recombination (HR) repair. The tumors show sensitive response to DNA damaging agents as synthetic lethality, so that a PARP inhibitor-based treatment has been provided in Japan. However, various types of acquired chemoresistance mechanisms were reported, resulting in treatment difficulty.

Therefore, we screened chemical compounds to explore novel factors to sensitize tumor cells combined with DNA damaging agents such as PARP inhibitor and etoposide. We utilized known compounds for drug-repositioning and performed our original high-throughput screening to assess DNA damage repair machinery. So far, we found Tioxolone one of carbonate dehydratase inhibitors as a PARP inhibitor sensitizer and Medroxyprogesterone acetate (MPA), one of steroids as an etoposide sensitizer. Each compound significantly increased DNA double-strand breaks (DSBs) and cytotoxicity with PARP inhibitor or etoposide, despite the compound alone did not induce DSBs. In addition, we showed that the synthetic lethal effect was caused by targeting a mechanism independent of DSB repair. These findings indicate that the sensitizers could induce effective synthetic lethality to tumors with acquired chemo-resistance via recovery of DNA repair capacity. Interestingly, each compound caused the synthetic lethality independently of its specific function, so that our research would be directed to drug repositioning. The detailed mechanisms are becoming clear and we will develop those for clinical applications.

Highlight

We had previously reported that BRCA2 is one of the factors that connect two centrosomes during the S phase. In fact, BRCA2 possesses a centrosomal localization signal (CLS) and a dominant-negative HA-CLS-DsRed fusion protein that inhibit the localization of BRCA2 at the centrosomes and result in the separation of centrosome pairs during the S phase. However, the physiological role of centrosome pairs during the S phase has not been clarified yet. Therefore, we conducted this study to clarify this issue. Our findings showed that the overexpression of HA-CLS resulted in the shortening of cilia extending from the centrosomes of MCF10A cells in the S phase compared with the overexpression of HA. Interestingly, aurora kinase A (AURKA), which is usually not localized to centrosomes during the S phase, was observed to localize to the centrosomes in cells treated with overexpression of HA-CLS, and the shortening of cilia was partially restored in the cells treated with siRNA-AURKA. As AURKA has been reported to degrade cilia in centrosomes, it is possible that the cilial degradation of centrosome separation during the S phase could be caused by AURKA recruitment to centrosomes. Therefore, we suggest that centrosome pairs play a vital role in maintaining cilia during the S phase. (**Figure**)
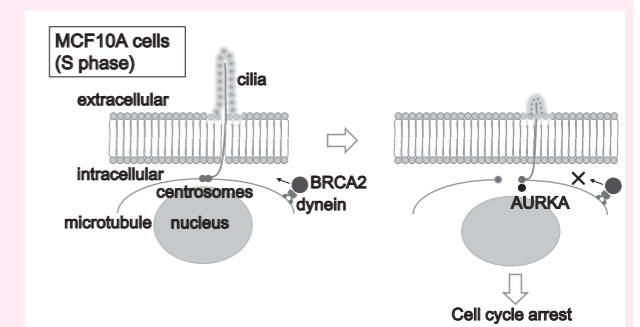
Physiological role of S phase centrosomes adjacency (model diagram)
BRCA2 localizes to the centrosome during S phase and connects the two centrosomes, at which time a primary cilium protrudes from one of the centrosomes. When BRCA2 was inhibited in the centrosomes, the centrosomes separated and AURKA, a degradation protein of primary cilia, localized in the centrosomes, resulting in shortening of primary cilia.

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[Original articles]
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3. Kaneyasu T…Miki Y, et al. Prevalence of disease-causing genes in Japanese patients with BRCA1/2-wildtype hereditary breast and ovarian cancer syndrome. NPJ Breast Cancer. 2020 Jun 12; 6:25. doi: 10.1038/s41523-020-0163-1.
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5. Torii S, Yamaguchi H, Nakanishi A, Arakawa S, Honda S, Moriwaki K, Nakano H, Shimizu S. Identification of a phosphorylation site on Ulk1 required for genotoxic stress-induced alternative autophagy. Nat Commun. 2020 Apr 9;11(1):1754. doi: 10.1038/s41467-020-15577-2.
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Department of Molecular Epidemiology

Professor	**Masaaki Muramatsu, M.D. & Ph.D.**
Associate Professor	**Noriko Sato M.D.& Ph.D.**
Assistant Professor	**Chihiro Imai, Ph.D.**

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 and study how epigenetics of the fetus and the mother are regulated.

1. Werner Syndrome Helicase (WRN) gene variants and cancer in Japanese elderly: An autopsy study

Werner Syndrome (WS) is a rare autosomal recessive disorder characterized by symptoms of premature aging, including elevated risk of malignancies. The causative WRN gene encodes a DNA helicase, which maintains the integrity of the human genome. While WS patients have functional null mutations in both alleles of the WRN gene, phenotypes of heterozygote carriers have not been described. Cellular assays showed that heterozygote carriers also have genetic instability to a lesser extent. To this end we searched for variants in the WRN gene among registered consecutive autopsy cases (n=2345, mean age=80) in the Japanese Geriatric SNP (JG-SNP) database, which includes detailed pathological documentation and exome chip analysis. The non-sense variant p.R369X (rs17847577), which is the second most prevalent mutation of WS in Japan was found in 5 heterozygotes (3 men and 2 women) ; the minor allele frequency (MAF) was 0.11%. The mean age of p.R369X heterozygotes was 89.8 years old, indicating that average length of life was attained. All three men had multiple primary cancers including lung cancer in common. One woman had thyroid cancer and the other had no cancer. These results suggest that p.R369X heterozygotes can expect average length of life, although men might be cancer prone. We also determined other non-synonymous variants p.V114I (MAF= 1.9%), p.E510D (0.68%), p.F1074L (33.38%) and p.C1376R (6.56%), with regard to cancer phenotypes in the database, and found that none of them were associated with presence of cancer in total. The information that p.

R369X carriers can expect average lifespan, would be important to whom this variant was incidentally found after personal genome sequencing.

2. D-amino acid oxidase (DAO) gene rare missense variant p.Pro103Leu and gastric cancer

Gastric cancer is a major type of cancer in Asian population. Genetic predisposition to gastric cancer is not fully understood. Recent studies have shown that D-amino acid oxidase (DAO), a multifunctional enzyme, protects the mucosa of gastrointestinal (GI) tracts by generating hydrogen sulfide (H_2S) in the stomach of rodents. We surveyed rare germline variants in the human DAO gene with regard to the incidence of gastric cancer. The consecutive autopsy cases registered in the JG-SNP database (n=2,343, mean age 80) were employed and genotyped with Exome BeadChips. There were three non-synonymous rare variants, p.R22H, p.P103L, and p.R283Q, of which the minor allele frequencies were 0.09%, 0.21% and 0.02%, respectively. Carriers of these variants were surveyed, and four out of ten p.P103L variant had gastric cancer (Fisher exact P=0.018). All four patients were men with drinking and smoking habits. Among the other six women, there were one small intestine cancer and one colon cancer. Neither p.R22H nor p.R283Q carriers had gastrointestinal cancer. DAO p.P103L is reported as a modifier of amyotrophic lateral sclerosis (ALS) and probably a hypomorphic allele. Thus, it is hypothesized that this rare variant might have affected the protective effect through H_2S signaling in the mucosa, which leads to high prevalence of gastric cancer. The role of rare variant DAO p.P103L warrants further investigation in larger cohorts.

3. DOHaD study

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. To implement preemptive medicine from the early stage of life, it is important to elucidate how the environment interacts with the fetal genome and modulates its phenotype. We are currently continuing our Birth Cohort – Gene and ENvironment Interaction Study of TMDU (BC-GENIST). Furthermore, we have initiated the collaboration with the ToMMo's Birth and Three-Generation Cohort and the Hamamatsu Birth Cohort groups, to further expand DOHaD research.

4. Single nucleotide polymorphisms in tinnitus patients exhibiting severe distress.

The association between distress caused by tinnitus and psychological factors such as depression and anxiety has been examined and reported. However, prognostic factors remain poorly understood because there are only a few reports on genetic associations. We theorized there might be an association between the grade of tinnitus distress and the genetic background related to psychological factors which might lead us to identify prognostic markers. We enrolled 138 patients who had suffered from tinnitus for over 3 months. Using Tinnitus Handicap Inventory (THI) scores, we examined the association between tinnitus distress and a genetic background related to depression or anxiety. A significant association between single nucleotide polymorphism rs131702 of the Breakpoint Cluster Region (BCR) gene and the severe THI score was identified. In addition, there was an association with the severity of the State-Trait Anxiety Inventory, an index of state anxiety severity. No association was found with the Self-Rating Depression Scale, an index of depression severity. It is reported that rs131702 of BCR in Japanese patients are related to bipolar II depression characterized by fluctuation between abnormal mood states of mania and depression. Our results indicate that rs131702 of BCR is independent of depression in this study and is, therefore, a prognostic factor unique to tinnitus. We conclude that the severity of tinnitus is associated with genes related to depression.

Publications

1. Minn AKK, Sato N, Mieno MN, Arai T, Muramatsu M. Association Study of Long Non-Coding RNA HOTAIR rs920778 Polymorphism With the Risk of Cancer in an Elderly Japanese Population. Gene 729:144263 (2020)

2. Abudushataer M, Sato N, Mieno M, Sawabe M, Muramatsu M, Arai T, Association of CYP2A6 gene deletion with cancers in the Japanese elderly: an autopsy study. BMC Cancer 20:186 (2020)

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4. Wang T, Matsuda Y, Nonaka K, Ishiwara T, Kanazawa N, Uewgai S, Muramatsu M, Sawabe M, Mori S, Tanaka M, Kitagawa M, Arai T. Clinicopathological characteristics of gastric cancer with CA19-9 expression occurring in elderly individuals: An autopsy study. Pathology International 70:92 (2020)

5. Zong Y, Tanaka M, Muramatsu M, Arai, T. Werner Syndrome Helicase (WRN) gene variants and cancer in Japanese elderly: An autopsy study J Geriatr Med Gerontol 6:103 (2020)

6. Zong Y, Tanaka M, Muramatsu M, Arai T. D-amino oxidase (DAO) gene rare missense variant p. Pro103Leu and gastric cancer. Mol & Clinc Oncology (2021) in press

7. Watabe T, Kanzaki S, Sato N, Matsunaga T, Muramatsu M, Ogawa K. Single nucleotide polymorphisms in tinnitus patients exhibiting severe distress. Sci Rep. 10:13023 (2020)

Department of Functional Genome informatics

Professor   Itoshi Nikaido, Ph.D.
Collaborative Researcher   Yohei Sasagawa, Ph.D.
Collaborative Researcher   Mariko Yamane, Ph.D.
Collaborative Researcher   Yoshimi Iwayama, MS.
Technical assistant   Ikuko Maeda

Research Objects

In this laboratory, we are developing new methods for large-scale genome experiments and data analysis techniques. We aim to realize drug discovery for intractable diseases and regenerative medicine by using these technologies. In recent years, much attention has been focused on understanding diseases from the cellular level, which is the smallest unit of life. We are developing a single-cell RNA-seq (scRNA-seq) method to measure the amount and type of RNA in each cell to measure cell function and condition in organs without exception. By analyzing the data on the amount and type of RNA per cell obtained by this method using artificial intelligence technology, we can identify cell function, differentiation lineage, and cell-cell interaction in organs. We are also developing algorithms and software to discover the causes of diseases and drug targets from scRNA-seq data using bioinformatics, machine learning, statistical science, and computer science. These technologies will contribute to developing drugs that target specific cells and regenerative medicine that supplements specific cells.

Research activities

(1) World's highest performance large-scale RNA-seq method: Quartz-Seq2

In this laboratory, we have developed Quartz-Seq2 (Sasagawa Y. et al. Genome Biol. 2018), a technology to measure the amount and type of RNA in a sample with high accuracy and high throughput. This technology showed the best results in the benchmarking study of scRNA-seq in the International Human Cell Atlas (HCA) project (Merue, et al. Nature Biotech. 2020).

Quartz-Seq2 performs a series of molecular biological reactions called whole transcript amplification (WTA) in vitro to detect small amounts of RNA derived from a single cell by DNA sequencer. WTA consists of two reaction steps: first, RNA is eluted from the cell and then reverse transcribed into cDNA. The second reaction is the amplification step of the cDNA molecules by PCR. We realized WTA by using the reaction principle of PolyA tailing reaction and suppression PCR. In particular, the PolyA addition reaction is more efficient than other methods. Therefore, the number of detected genes by Quartz-Seq2 is superior to other methods. We have incorporated cell barcoding and multiplex reactions to enable Quartz-Seq2 to be performed on large numbers of cells. Quartz-Seq2 can perform thousands or tens of thousands of scRNA-seqs with high accuracy.

An international research project, Human Cell Atlas, is currently underway to analyze and compile an atlas of all cell functions that make up the human body using scRNA-seq. In this project, various partners are conducting scRNA-seq analysis of different human organs, integrate all the data from multi-research center, and provide the data to researchers via the Internet. To integrate data from all sequencing facilities, we should understand the performance difference of the scRNA-seq method used at each facility and confirm whether data integration is possible. Therefore, HCA conducted a study to compare the performance of scRNA-seq methods. First, cell suspensions containing a variety of human and mouse cell types were divided into equal samples and frozen and transported to the developers of the scRNA-seq method and analysis facilities around the world. All facilities analyzed the samples using each scRNA-seq at each site, and the sequence data were submitted to the data analysis sites via the Internet. The performance comparison team calculated six performance indices and compared the performance of each method after arranging the amount of data so that the data could be compared fairly. As a result, Quartz-Seq2 showed the highest performance in the overall score integrating the six performance indices. The number of detected genes was 1.5 to 4 times higher than that of the other methods.

(2) Elucidation of disease mechanisms using large-scale RNA-seq and disease-specific iPS cell

In our laboratory, we also aim to elucidate the mechanism of diseases from the cellular level, the smallest unit of life, using Quartz-Seq2. In this year, we have clarified the mechanism of psychiatric disorders. First, elucidating the mechanisms of psychiatric disorders is problematic because it requires studying the human brain. Therefore, our collaborator established patient-derived pluripotent stem cells (iPS cells) and generated a cell mass (brain organoid) that resembles human brain tissue in vitro (Sawada T. et al. Mol. Psychiatry. 2020). The organoid contains various neurons and other nerve cells that make up the brain, and these cells inherit the congenital and acquired genetic information of the patient. Here, we generated brain organoids from human iPSC cells derived from monozygotic twins who do not share the same onset of psychiatric disorders. By comparing the properties of these cells, we can analyze the mechanism by which the disease develops despite the identical genetic background. To investigate the cellular functions and states of these brain organoids, we performed scRNA-seq using Quartz-Seq2. The results showed that patient-derived brain organoids are prone to become inhibitory GABAergic neurons. Furthermore, we found that the reason for the increase in the number of these cells was related to Wnt signal. The addition of Wnt signaling to patient-derived cells reduced the difference in cell differentiation ability. These results suggest that the onset of psychiatric disorders may be caused by a decrease in Wnt signaling for some reason during development. This research was conducted by Dr. Tadafumi Kato and colleagues at the Laboratory for Dynamics of Mental Disorders, RIKEN Brain Science Institute.

Research achievements

Original Paper
1. Tomoyo Sawada, Thomas E. Chater*, Yohei Sasagawa*, Mika Yoshimura, Noriko Fujimori-Tonou, Kaori Tanaka, Kynon J. M. Benjamin, Apuã C. M. Paquola, Jennifer A. Erwin, Yukiko Goda, Itoshi Nikaido, Tadafumi Kato. Developmental Excitation-Inhibition Imbalance Underlying Psychoses Revealed by Single-Cell Analyses of Discordant Twins-Derived Cerebral Organoids. Molecular Psychiatry. 2020 (*These authors contributed equally)
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Department of Genomic Function and Diversity

Professor   Yuta Kochi
Associate professor   Satomi Mitsuhashi
Assistant professor   Mahoko Ueda

Research objectives

Complex diseases such as immunological diseases, metabolic diseases, dementia, and cancers are caused by both genetic and environmental factors, with varying combinations in different individuals. Genome-wide association studies (GWAS) have led to the discovery of thousands of risk variants involved in these diseases, but the precise mechanisms of the diseases are not fully understood. Our laboratory aims to elucidate the disease etiology by dissecting the diversity of genomic function among individuals. To this end, we integrate bioinformatic approaches with molecular biology techniques in the analysis of genetic variants such as expression quantitative trait locus (eQTL) and splicing QTL (sQTL) mapping. We will also establish to predict each individual's pathophysiology (disease severity, drug response, etc.) based on the individual's genome information to bring precision medicine into clinical practice.

Research activities

1. Integration of GWAS and eQTL/sQTL studies

Majority of GWAS loci identified in complex traits are now considered to be eQTL or sQTL where genetic variants regulate expression levels of genes or alternate splicing. Therefore, to interpret the results of GWAS for dissecting the mechanism of disease, it is essential to integrate the results of GWAS and eQTL/sQTL studies. Several international projects such as gEuvadis project and GTEx project have been performed to identify eQTL and sQTL in multiple types of cells and tissues. We have also performed an QTL study for immune cell subsets to establish eQTL catalog in Japanese (*Nat Genet* 2017). By combining those eQTL data with disease GWAS data, we have successfully identified many disease-associated eQTL. However, because some of eQTL effects are observed in more cell-specific manners or only in stimulated cells, further analysis is needed to more precisely dissect the roles of disease-relevant cells in the disease etiology.

Meanwhile, sQTL is another major cause of complex diseases. Whereas eQTL changes the gene function quantitatively, alternative splicing may change the protein function qualitatively. Thus, the sQTL study may directly indicate the disease-causing isoform that offers clues to the mechanism of disease. However, because conventional "short-read" sequencing cannot reveal the whole transcript sequences, sQTL study using the short-read sequencing provide limited information on the transcripts. We will utilize long-read sequencing such as Nanopore or PacBio technologies to complement existing sQTL studies.

2. Functional analysis of GWAS genes

GWASs have identified over 100 susceptibility gene loci per disease. Although the contribution of each genetic factor to entire genetic factors is small, it does in fact form one aspect of the pathology. Clarifying the function of individual susceptibility genes is the first step in elucidating the pathology. Indeed, we have shown that in GWAS of clinically amyopathic dermatomyositis (CADM), a genetic variant having sQTL effect on the *WDFY4* gene is associated with the disease. Increased spliced-isoform observed in the risk allele produced a C-terminal-deficient WDFY4 protein, and this protein enhanced the signal of MDA5, an RNA virus recognition receptor (*Ann Rheum Dis* 2018). Since CADM complicates rapidly progressive interstitial pneumonia with a fatality rate of 50%, a therapy targeting this C-terminal-deficient WDFY4 protein would be promising in this fatal condition. This example of *WDFY4* locus may indicate a detailed functional analysis of the GWAS candidate locus can provide knowledge directly related to treatment of a disease.

On the other hand, the advent of long-read sequencers has revealed that structural variants and repetitive sequence variants contribute to various diseases. These genetic variants may be causative in some GWAS loci. Furthermore, some RNAs transcribed from dispersed repetitive sequences derived from retrotransposon function in innate immunity, and they are involved in the pathophysiology of not only immune diseases but also other multifactorial diseases such as Alzheimer's disease. We therefore analyze the function of these unexplored genomic sequences by utilizing long-read sequencing technologies.

3. Analysis of functional genetic variants and disease mechanism by systems approaches

The analysis of individual genetic factors reveals one aspect of the pathogenesis of multifactorial diseases, but it is the accumulation of these genetic factors that forms the overall pathology. Therefore, it is necessary to analyze the stacking of genetic factors using a systems approach, assuming that the disease is a system, in order to evaluate the overall picture of the disease state and its differences between individuals. Indeed, we have shown that eQTL stacking in CD4-positive T cells contributes to TNF-α activation by assessing the genome of rheumatoid arthritis patients (*Nat Genet* 2017). We aim to establish a method for predicting disease states using genomic information by integrating various omics data such as GWAS, eQTL, and sQTL. In addition, with the cooperation of clinicians and disease cohorts, we will verify our predictive models to realize precision medicine in clinic.

Publications

Original articles
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2. Inamo J, Kochi Y, Takeuchi T. Is type 2 diabetes mellitus an inverse risk factor for the development of rheumatoid arthritis? J Hum Genet. 66. 219-23, 2021.
3. Frith MC, Mitsuhashi S, Katoh K. lamassemble: Multiple Alignment and Consensus Sequence of Long Reads. Methods Mol Biol. 2231. 135-45, 2021.
4. Bing N, Zhou H, Chen X, Hirose T, Kochi Y, et al. Contribution of a European-Prevalent Variant near CD83 and an East Asian-Prevalent Variant near IL17RB to Herpes Zoster Risk in Tofacitinib Treatment: Results of Genome-Wide Association Study Meta-Analyses. Arthritis Rheumatol. 2021.
5. Yin X, Kim K, Suetsugu H, Bang SY, Wen L, et al. Meta-analysis of 208370 East Asians identifies 113 susceptibility loci for systemic lupus erythematosus. Ann Rheum Dis. 2020.
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7. Otomo A, Ueda MT, Fujie T, Hasebe A, Suematsu Y, et al. Efficient differentiation and polarization of primary cultured neurons on poly(lactic acid) scaffolds with microgrooved structures. Sci Rep. 10. 6716, 2020.
8. Mitsuhashi S, Ohori S, Katoh K, Frith MC, Matsumoto N. A pipeline for complete characterization of complex germline rearrangements from long DNA reads. Genome Med. 12. 67, 2020.
9. Ishigaki K, Akiyama M, Kanai M, Takahashi A, Kawakami E, et al. Large-scale genome-wide association study in a Japanese population identifies novel susceptibility loci across different diseases. Nat Genet. 52. 669-79, 2020.
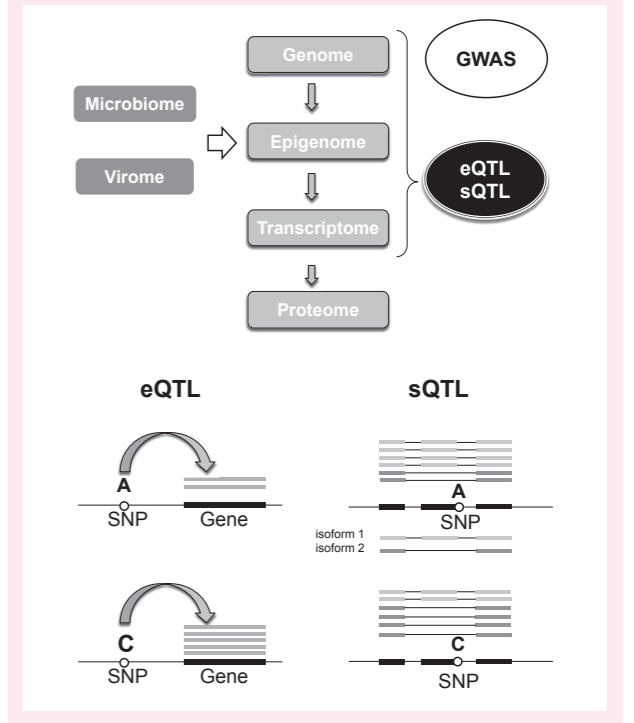
Review articles
1. Kochi Y. Genomic analysis of human immunological diseases (in Japanese). Inflammation and Immunity. 28. 460-464, 2020

Department of Epigenetics

Professor	Fumitoshi ISHINO
Associate Professor	Lee JIYOUNG
Assistant Professor	Moe KITAZAWA
Project Assistant Professor	Ayumi MATSUZAWA
Adjunct lecturer	Takashi KOHDA, Shin KOBAYASHI, Hirosuke SHIURA, Taeko NARUSE

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. Certain mammalian-specific genes from LTR retrotransposons/retroviruses are essential for mammalian development, such as placenta and brain functions. Organoids from mouse and human ES and/or iPS cells mimic normal *in vivo* development. These studies show us how Epigenetics and Genetics are important in mammalian and human biology. We focus on these mammalian- as well as primate-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. Genomic imprinting diseases caused by deficiency and overexpression of *PEG11/RTL1* (see Highlight 1)

We have recently identified *PEG11/RTL1* is a major responsible gene for Kagami-Ogata and Temple syndromes as an essential muscle gene in a fetal and neonatal period-specific manner (Kitazawa *et al*. Development 2020) as well as an important brain gene, possibly contributed to eutherian brain evolution (Kitazawa *et al*. Genes Cells 2021).

2. Roles of retrovirus-derived genes in mammalian development and evolution

We have been carrying out a comprehensive analysis on retrovirus-derived genes, called Retrotransposon Gag like/sushi-ichi-related retrotransposon homologue (*RTL/SIRH*) genes by collaboration with Prof Kaneko-Ishino at Tokai University. We have demonstrated that *Peg10*, *Peg11/Rtl1* and *Sirh7/Ldoc1* are essential placental genes in eutherians, while *Sirh3/Ldoc1l* and *Sirh8/Rgag4* have some important functions in the brain (Irie *et al*. submitted) in addition to *Sirh11/Zcchc16* and *Peg11/Rtl1*. It is probable that these genes have contributed to brain evolution in eutherians.

3. New method for generation of heart-origanoid (see Highlight 2)

We have developed a new method for generating heart-organoid (HO) from mouse ES cells (Lee *et al*. Nat Commun 2020) as well as human iPS and ES cells. The generated HO mimics embryonic heart development and has both atria and ventricle parts. It exhibits beating like *in vivo* heart, therefore, is a promising tool for analyzing normal heart development and examining heart toxicity of newly developed drugs.

4. A new method for detecting unidentified retrovirus-derived genes in the human genomes

Approximately 8 % of the human genome is occupied by LTR retrotransposons/retroviruses. Most were inactivated by a multiple of mutations, however, there exist more than 10,000 open reading frames that can potentiaiiy encode >100 amino acid proteins. It remains elusive that they could function as *bona fide* protein-coding genes. We have developed a novel system to detect retrovirus-derived genes with a high degree of sensitivity during *in vitro* differentiation/organoid formation from human iPS cells (Matsuzawa *et al*. Int J Med Sci 2021).

Fetal muscle development and *PEG11/RTL1*

Temple and Kagami-Ogata syndromes are human genomic imprinting diseases that caused by maternal and paternal disomy of chromosome 14 (Upd(14)mat and upd(14)pat), respectively. They exhibit certain muscle and brain related symptoms, such as muscle hypotonia, feeding difficulty/poor sucking function in early childhood, speech delay and slight intellectual defects in the former, in contrast to neonatal lethality due to respiratory problems associated with a bell-shaped thorax, abdominal wall hernia and severe intellectual defects in the latter (Fig. 1). We have previously demonstrated that mouse *Peg11/Rtl1* is one of the major genes responsible for the placental abnormalities in these two syndromes. Recently, we detected severe but distinct abnormalities in the several neonatal muscles that are essential for respiration, such as the intercostal, abdominal and diaphragm muscles in both cases (Fig. 2, upper panel). PEG11/RTL1 protein localizes around the Z-disc in muscle fiber interacting the DESMIN protein that is essential for skeletal muscle stability and force generation (Fig. 2, lower panel), suggesting the role of PEG11/RTL1 for stabilizing muscle fiber. We propose an evolutionary role of the domestication of *PEG11/RTL1* in fetal/neonatal muscle development in terms of presumable adaptation to viviparous reproduction system in eutherian mammals (Kitazawa *et al*. Development 2020). In addition, PEG11/RTL1 is expressed in corticospinal tract and corpus callosum, the mammalian- and eutherian-specific brain structures (Fig. 3) and its under- and over-expression causes causes motor function defects and increased anxiety in mice, indicating *PEG11/RTL1* is also responsible for several neurological symptoms in these diseases. Moreover, it is likely that the acquisition of *PEG11/RTL1* was deeply involved in eutherian brain evolution.
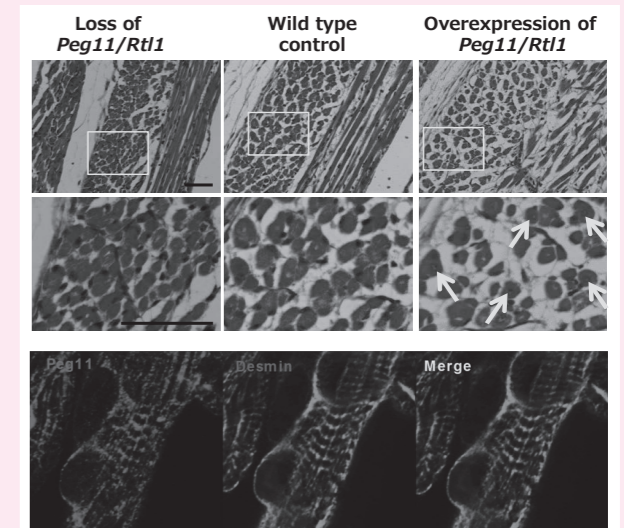
Figure 2. Muscle abnormalities in loss and overproduction of *Peg11/Rtl1*.
Upper columns: HE staining of neonatal intercostal muscle. Middle columns: a more highly magnified view of the yellow boxes in the upper column. *Peg11/Rtl1* KO (left), wild type (center) and *Peg11/Rtl1* overproduction mice (right). Scale bars, 1 mm (upper) and 50 μm (middle), respectively. Lower columns: Immunofluorescence staining of the PEG11/RTL1 and DESMIN proteins in the neonatal abdominal muscles from Mat-KO mice. Scale bars, 20 μm.
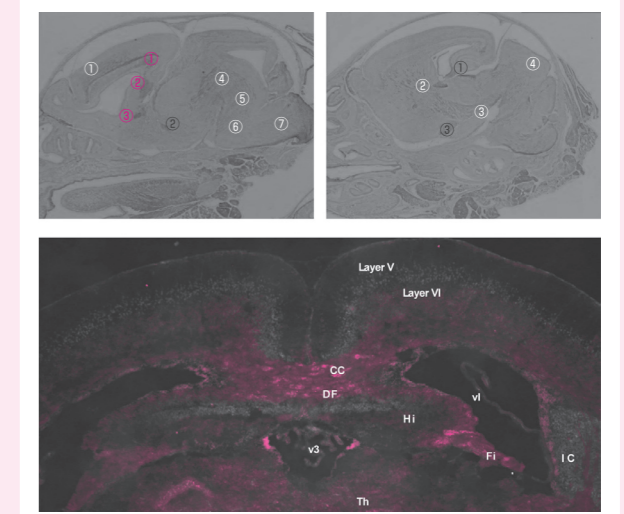
Figure 3. Expression of PEG11/RTL1 protein in the brain.
Upper columns (sagittal sections): The PEG11/RTL1 protein is detected in descendent tracts including corticospinal tract (①~⑦ in white), commissure neurons, such as corpus callosum, hippocampal and anterior commissures (①~③ in red) and limbic systems, such as hippocampal fimbria, fornix and medial amygdala nucleus (①~③ in gray). Lower column (coronal section), CC: corpus callosum, DF: dorsal fornix, Hi: hippocampus, Fi: fimbria, IC: internal capsule, Layer V and VI: cerebral cortex V and VI layers, Th: thalamus, V3: the third ventricle, VI: lateral ventricle.
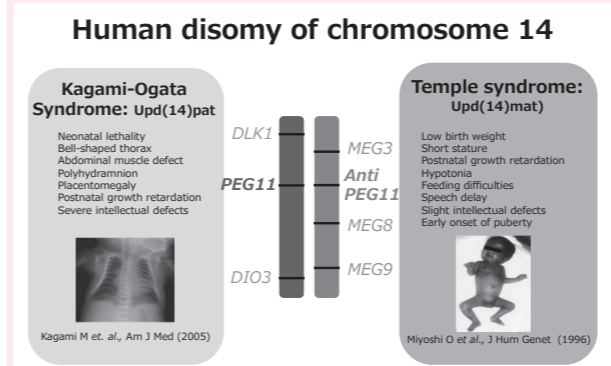
Human disomy of chromosome 14

Figure 1. Kagami-Ogata syndrome and Temple syndrome.
The features of Kagami-Ogata syndrome (Left) and those of Temple syndrome (right). Both exhibit muscle- and brain related defects.

Functional heart organoids from mouse ES cells

The heart comprises multiple layers of tissue including many different cell types, including cardiomyocytes, smooth muscle cells and endothelial cells. These cells work together to ensure a proper functioning of the heart and thus the constant supply of fresh, oxygenated blood to the rest of the body. Studying all forms of heart disease in the laboratory and developing novel drugs to treat these diseases require disease models that closely resemble the actual heart. We succeeded to generate functional heart organoids from mouse embryonic stem cells by being exposed to FGF4 and LN/ET (Fig. 4). Importantly, they showed considerable similarity to the developing heart based on structural as well as molecular analyses. We also succeeded to generate similar functional heart organoids from human iPS and ES cells. They would be ideal tool for drug sensitivity screening, such as car-diac toxicity as well as identification and biological analysis of as-yet-unidentified retrovirus-derived genes in the human genomes as mentioned in 4.
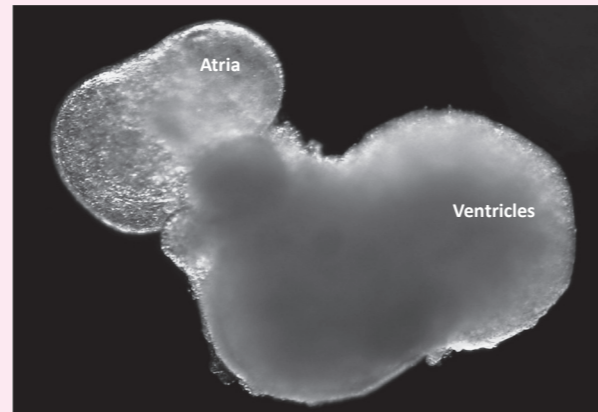

Figure 4. Mouse heart organoid with atria and ventricles
The first achievement of generating functional heart organoid with proper conduction system in the world.

Publications

Original papers
1. Kitazawa M, Hayashi S, Imamura H, Takeda S, Oishi Y, Kaneko-Ishino T* and Ishino F*. Deficiency and overexpression of *Rtl1* in the mouse cause distinct muscle abnormalities related to the Temple and Kagami-Ogata syndromes. Development **147**(21):dev185918 (2020). doi: 10.1242/dev.185918.
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3. Kitazawa M, Sutani A, Kaneko-Ishino T* and Ishino F*. The role of eutherian-specific *RTL1* in the nervous system and its implications for the Kagami-Ogata and Temple syndromes. Genes Cells **26**:165-179 (2021). doi: 10.1111/GTC.12830

Department of Medical Science Mathematics

Professor   Tatsuhiko Tsunoda
Junior Associate Professor   Fuyuki Miya

Research Summary

Effective utilization of rapidly developing omic profiling technologies and, in particular, the introduction of personalized/precision/preventive medicine have recently become major goals of medical research. Our laboratory develops strategies to address these challenges by bringing the ideas and methods from mathematics and computational sciences to the medical domain. The first part of our approach is driven by integrative analysis of clinical and omic data and aims to explore the etiologies of intractable diseases such as cancer, common diseases, and neurodegenerative diseases. Next, we classify each disease into more refined categories using molecular profiles and clarify the underlying causal mechanisms using systems-based approaches. Lastly, we apply mathematical and machine learning techniques to infer optimal therapy for each patient to guide treatment decisions by their hospital or clinic.

Research Projects

1. Pan-cancer analyses of whole genomes

Participating in the International Cancer Genome Consortium (ICGC), we analyzed the whole genome of 2,658 cases across all cancer types for the effects of both coding and non-coding genomic regions on cancer. As a result, we found significant effects of mutations in long non-coding RNA and gene-regulatory regions. By cataloging and analyzing the functions of these genes, we have established a basis for new drug discovery for various types of cancer [1-24].

2. Estimation of the number of simultaneous metastatic cells during cancer metastasis

In order to elucidate how many cells from primary tumors could be contained in metastatic tumors, we developed a mathematical method to quantify the "founder cell population size" at the metastasis site using WES data of both the tumors. By applying our method to real data from colorectal cancer patients, we found metastases of populations of 3-17 cells. This phenomenon may have been cause of differences in treatment responses [25].

3. Post-translational modification and protein-structure prediction

Recently, we have developed prediction models for post-translational amino-acid modifications and protein structures using various physicochemical and sequence properties [26-29].

4. Whole exome sequence (WES) analysis for mendelian disease

One type of next-generation sequencing data analyses is WES analysis. This approach has made a particularly strong impact in the studies of Mendelian diseases using family data. We have identified many disease-causing genes of intractable diseases, e.g. neurodegenerative diseases, by using our original experimental methods and analysis pipeline that were developed to achieve both high coverage and accuracy [30].

5. Results of other research projects [31-36]

Publications

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Frontier Research Unit
Laboratory of Gene Expression

Associate Professor   Hidehito KUROYANAGI

Laboratory for Integrated Research Projects on Intractable Diseases
Advanced Technology Laboratories

Post-transcriptional regulation is an important layer for gene expression regulation. Most of human multi-exon genes produce multiple mRNA isoforms through alternative pre-mRNA processing and hence multiple structurally and functionally distinct protein isoforms in cell-type-specific manners. We are trying to decipher so-called "cellular codes" that determine the cell-type specific pre-mRNA processing patterns and to elucidate molecular basis for pathogenesis of genetic diseases caused by defects in post-transcriptional regulation of gene expression.

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

We developed a transgenic fluorescence alternative splicing reporter system that enabled visualization of alternative pre-mRNA processing patterns *in vivo* (Nat Meth, 2006; Nat Protoc, 2010). With the reporter system, we have visualized spatiotemporal profiles of alternative pre-mRNA processing events in a living nematode worm *C. elegans* and identified trans-acting factors and *cis*-elements involved in the regulation (Mol Cell Biol, 2007; Genes Dev, 2008; PLoS Genet, 2012, 2013; NAR, 2013, 2016; Nat Struct Mol Biol, 2014; Nat Commun, 2016; Mol Cell Biol, 2018; Cytoskeleton, 2018). Through these studies, we now realize that molecular mechanisms for post-transcriptional gene regulation are conserved throughout metazoan evolution (WIREs RNA, 2017; Genetics, 2020).

Animal Models for Dilated Cardiomyopathy and Arrhythmia.

Dilated cardiomyopathy (DCM) is a disease in which the heart becomes enlarged and no longer pumps blood effectively. Autosomal-dominant familial DCM is linked to mutations in a variety of genes including a splicing regulator RBM20. We reported for the first time that phosphorylation of the two serine residues in an RSRSP stretch, a hotspot of the DCM mutations in the *RBM20* gene, were essential for nuclear localization, which allows RBM20 to interact with its target pre-mRNAs (Sci Rep, 2018; Front Mol Biosci, 2018). We generated an *Rbm20*^{S637A} knock-in mouse, mimicking an un-phosphorylatable mutation found in a well-studied case of DCM as well as an *Rbm20* knock-out mouse, and confirmed that the serine residue is critical for the splicing regulation by RBM20 in the heart (Sci Rep, 2018, 2020). Surprisingly, only the *Rbm20*^{S637A} knock-in mouse developed DCM-like phenotypes and fatal arrhythmia (Figure, Sci Rep, 2020). Similar DCM-like phenotypes were also observed in a pig model of DCM caused by another missense mutation in the RSRSP stretch (Nat Med, 2020). The knock-in animal models will be excellent new tools to elucidate mechanisms of DCM pathogenesis and to develop effective therapeutics.
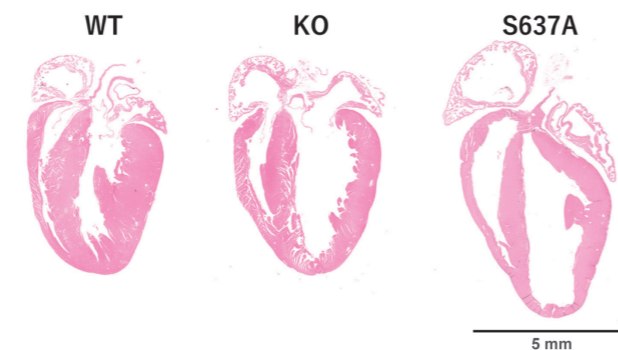
WT	KO	S637A

5 mm

Figure. Morphology of the hearts from *Rbm20*-gene modified mice.

Publications

Original Articles
1. Kensuke Ihara, Tetsuo Sasano, Yuichi Hiraoka, Marina Togo-Ohno, Yurie Soejima, Motoji Sawabe, Megumi Tsuchiya, Hidesato Ogawa, Tetsushi Furukawa, Hidehito Kuroyanagi. A missense mutation in the RSRSP stretch of *Rbm20* causes dilated cardiomyopathy and atrial fibrillation in mice. **Scientific Reports**, 10: 17894, 2020.
2. Jay W Schneider, Saji Oommen, Muhammad Y Qureshi, Sean C Goetsch, David R Pease, Rhianna S Sundsbak, Wei Guo, Mingming Sun, Han Sun, Hidehito Kuroyanagi, Dennis A Webster, Alexander W Coutts, Kimberly A Holst, Brooks S Edwards, Nikolas Newville, Matthew A Hathcock, Tamene Melkamu, Francesca Briganti, Wu Wei, Maria G Romanelli, Scott C Fahrenkrug, Doug E Frantz, Timothy M Olson, Lars Steinmetz, Daniel F Carlson, Timothy J Nelson, Wanek Program Pre-Clinical Pipeline. Dysregulated RNP granules promote cardiomyopathy in *RBM20* gene-edited pigs. **Nature Medicine**, 26: 1788–1800, 2020.

Review Article
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Systems Biology for Intractable Diseases

It is getting clearer that pathogenesis of intractable disease is a state that deviates from an integrated system's control to abnormal situation where multiple genes are affecting one another intricately. The recent advances in biomedical research have been producing large-scale, ultra-high dimensional, ultra-heterogeneous omics data through the advanced technologies such as genome sequencing and proteome analysis. Also, not only our own genome, but also dysbiosis of commensal microbiota is known to be related with various diseases including IBD, Parkinson disease and other intractable diseases. The aim

of this section is to clarify the biological mechanisms and their failures in the system by applying computational strategy for systems biology and by analyzing these omics data using supercomputer. It is expected that key molecules of the diseases will be searched by the systems biology analysis of molecular pathways and networks related to the diseases which could not be analyzed in the traditional approaches. This section is collaborating with various laboratories in Medical Research Institute for understanding the pathogenesis of the diseases toward drug discovery and new therapy development.

Algorithm for Optimal
HC+BS Algorithm
Whole-Genome NNSR Algorithm
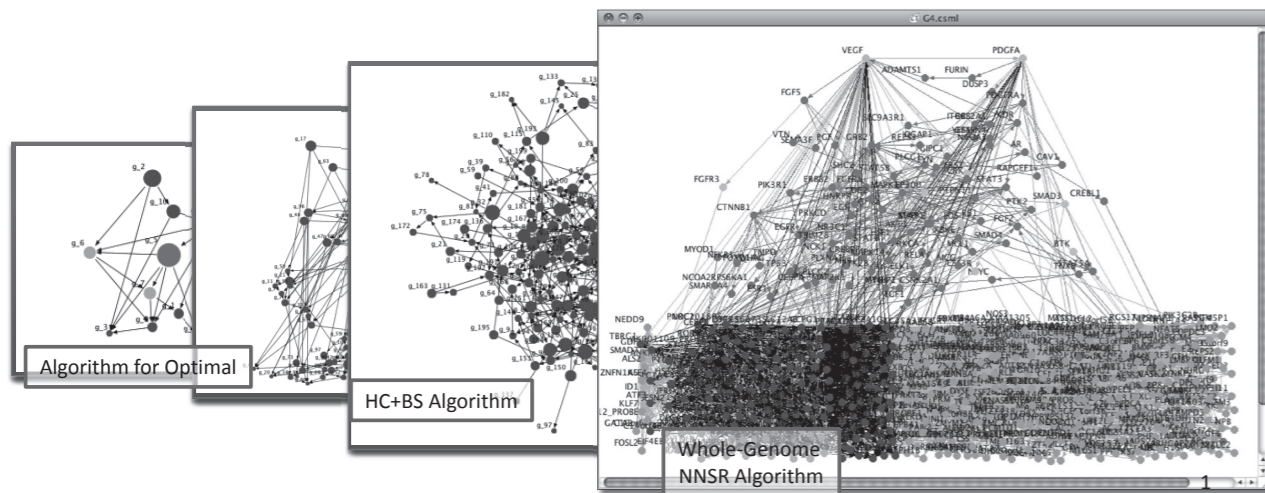
We have developed a series of programs on supercomputers for mining gene networks of size from 30 (optimal) to 20,000 (genome-wide) including non-coding RNAs based on various mathematical models (Bayesian N, State Space Model, Structural EQ, etc.). These programs are open-access with source codes.
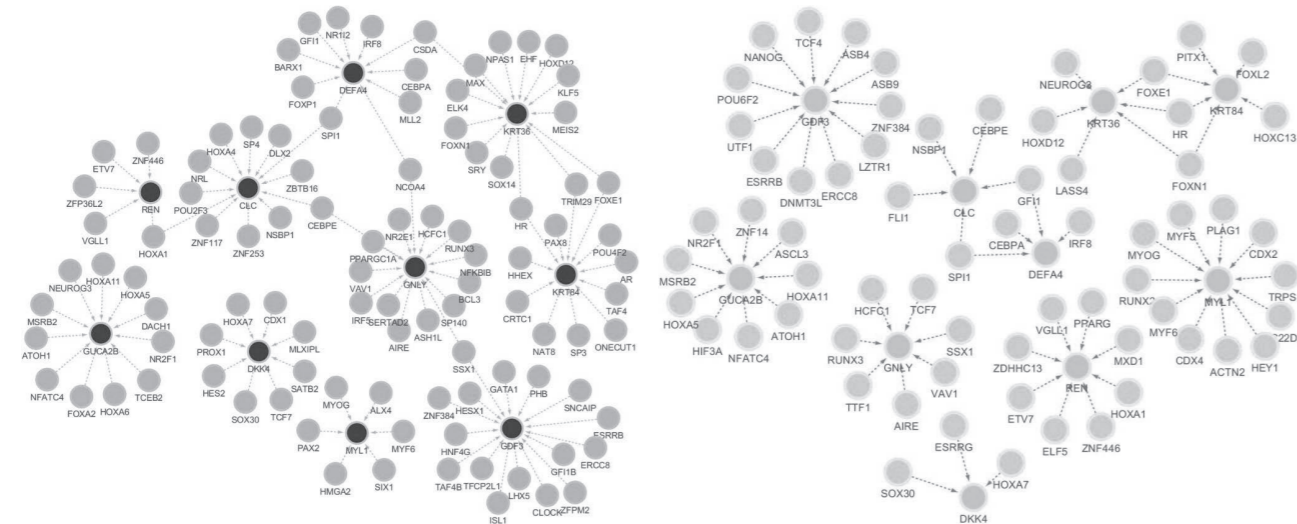
Gene network for the anticancer drug-resistant sample (left) and gene network for the anticancer drug-sensitive sample estimated from Sanger Genomic of Drug Sensitivity in Cancer data by the method of Park et al. J Comp Biol. 2019.

Publications

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IBD project3 Laboratory for Integrated Research Projects on Intractable Diseases

Principal Researcher   Shigeomi SHIMIZU
Research collaborators   Toshiaki OHTEKI
Satoko Arakawa
Taku Sato
Yoichi Nibe

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

Result 1. In order to elucidate the relationship between alternative autophagy and IBD, we generated various types of intestine-specific alternative autophagy knockout mice. Some mice were susceptible to DSS-induced colitis, suggesting protective role of alternative autophagy on colitis.

Result 2. In alternative autophagy machinery, we identified crucial role of Ulk1 phosphorylation at Serine476. We also discovered Wipi3 as an essential molecule for alternative autophagy.

Result 3. We identifies *Sanguisorba officinalis L.* derived from herbal medicine prevents intestinal inflammation by inducing autophagy in macrophages

Result 4. The small intestine has a robust regenerative capacity, and various cell types serve as "cells-of-origin" in the epithelial regeneration process after injury. However, how much each population contributes to regeneration remains unclear. Using lineage tracing combined with single-cell qRT-PCR and RNA-seq analysis, we found that CD81^{hi}Sca1$^-$ Lgr5-derivatives are the major cell of origin for regeneration in the damaged mouse intestine (Scientific Reports 2020).

Result 5. Intestinal stem cells (ISCs) are located at the crypt base and fine-tune the balance of their self-renewal and differentiation, but the physiological mechanism involved in regulating that balance remains unknown. We found that interferon regulatory factor-2 (IRF2), a negative regulator of interferon (IFN) signaling, preserves the stemness of ISCs by restricting their differentiation into secretory-cell lineages (Nature Cell Biology 2020).

Result 6. Using a dextran sodium sulfate (DSS)-induced colitis model, we explored that chronic IFN signaling physiologically stresses colonic epithelial stem cells (CoSCs) and IRF2 maintains the stemness of COSCs by limiting the physiological stress from IFN (Scientific Reports 2020).

Publications

1. Identification of a phosphorylation site on Ulk1 required for genotoxic stress-induced alternative autophagy. S. Torii, H. Yamaguchi, A. Nakanishi, S. Arakawa, S. Honda, K. Moriwaki, H. Nakano, S. Shimizu Nature Commun 11, Article number: 1754 (2020)
2. Sanguisorba officinalis L. derived from herbal medicine prevents intestinal inflammation by inducing autophagy in macrophages. A. Yasueda, H. Kayama, M. Murohashi, J. Nishimura, K. Wakame, K. Komatsu, T. Ogino, N. Miyoshi, H. Takahashi, M. Uemura, C. Matsuda, T. Kitagawa, K. Takeda, T. Ito, Y. Doki, H. Eguchi, S. Shimizu, T. Mizushima. Scientific Reports 10, Article number: 9972 (2020)
3. Wipi3 is essential for alternative autophagy and its loss causes neurodegeneration. H. Yamaguchi, S. Honda, S. Torii, K. Shimizu, K. Katoh, K. Miyake, N. Miyake, N. Fujikake, H. Sakurai, S. Arakawa, S. Shimizu. Nature Commun 11, Article number: 5311 (2020)
4. Characterization of radioresistant epithelial stem cell heterogeneity in the damaged mouse intestine. T. Sato, S. M Sase, S. Ishikawa, M. Kajita, J. Asano, T. Sato, Y. Mori, T. Ohteki. Scientific Reports 10, Article number: 8308 (2020)
5. Regulated IFN signaling preverves the stemness of intestinal stem cells by restricting differentiation into secretory-cell lineages. T. Sato, S. Ishikawa, J. Asano, H Yamamoto, M. Fujii, T. Sato, K. Yamamoto, K. Kitagaki, T. Akashi, R. Okamoto, T. Ohteki. Nature Cell Biology 22, Article number: 919 (2020)
6. IRF2 maintains the stemness of colonic stem cells by limiting physiological stress from interferon. K. Minamide, T. Sato, Y. Nakanishi, H. Ohno, T. Kato, J. Asano, T. Ohteki. Scientific Reports 10, Article number: 14639 (2020)

Research Project on Heart Diseases

Principal Researcher   Hidehito KUROYANAGI
Research collaborators   Tetsushi FURUKAWA
Kensuke IHARA
Yuichi HIRAOKA
Tetsuo SASANO

Model animals for Dilated Cardiomyopathy and Arrhythmia

Dilated cardiomyopathy (DCM) is a heart disease characterized by left ventricular dilatation and systolic dysfunction. Recent genetic studies on DCM have identified causative mutations in over 60 genes, including *RBM20*, which encodes a regulator of heart-specific splicing. DCM patients with *RBM20* mutations have been reported to present with more severe cardiac phenotypes, including impaired cardiac function, atrial fibrillation (AF), and ventricular arrhythmias leading to sudden cardiac death. We previously reported that an RSRSP stretch of RBM20, a hotspot of missense mutations found in patients with idiopathic DCM, functions as a crucial part of its nuclear localization signals (Sci Rep, 2018; Front Mol Biosci, 2018).We found that *Rbm20* mutant mice harboring a missense mutation S637A in the RSRSP stretch, mimicking that in a DCM patient, demonstrated severe cardiac dysfunction and spontaneous AF and ventricular arrhythmias mimicking the clinical state in patients. In contrast, *Rbm20* mutant mice with frame-shifting deletion demonstrated less severe phenotypes, although loss of RBM20-dependent alternative splicing was indistinguishable. The *Rbm20*^{S637A} mutant mice will be excellent and useful animal models for DCM and arrhythmia.

Publications

[Original Articles]
1. Kensuke Ihara, Tetsuo Sasano, Yuichi Hiraoka, Marina Togo-Ohno, Yurie Soejima, Motoji Sawabe, Megumi Tsuchiya, Hidesato Ogawa, Tetsushi Furukawa, Hidehito Kuroyanagi. A missense mutation in the RSRSP stretch of *Rbm20* causes dilated cardiomyopathy and atrial fibrillation in mice. Scientific Reports, 10: 17894, 2020.
2. Jay W Schneider, Saji Oommen, Muhammad Y Qureshi, Sean C Goetsch, David R Pease, Rhianna S Sundsbak, Wei Guo, Mingming Sun, Han Sun, Hidehito Kuroyanagi, Dennis A Webster, Alexander W Coutts, Kimberly A Holst, Brooks S Edwards, Nikolas Newville, Matthew A Hathcock, Tamene Melkamu, Francesca Briganti, Wu Wei, Maria G Romanelli, Scott C Fahrenkrug, Doug E Frantz, Timothy M Olson, Lars Steinmetz, Daniel F Carlson, Timothy J Nelson, Wanek Program Pre-Clinical Pipeline. Dysregulated RNP granules promote cardiomyopathy in *RBM20* gene-edited pigs. Nature Medicine, 26: 1788–1800, 2020.
3. Jiyoung Lee, Akito Sutani, Rin Kaneko, Jun Takeuchi, Tetsuo Sasano, Takashi Kohda, Kensuke Ihara, Kentaro Takahashi, Masahiro Yamazoe, Tomohiro Morio, Tetsushi Furukawa & Fumitoshi Ishino. In vitro generation of functional murine heart organoids via FGF4 and extracellular matrix. Nature Communications 11: 4283 (2020).

Molecular analysis of malignant breast cancer development under hypoxic condition

Principal Researcher   Koh Nakayama
Project collaborators   Hiroshi Shibuya
Yoshio Miki
Fumitoshi Ishino

Outline

Tumor microenvironment is often hypoxic, and induces malignant transformation of cancer cells. Breast cancer patients are increasing every year in women worldwide. Thus, it is important to understand how hypoxic environment affects breast cancers. Breast cancers are treated based on their subtypes, and there are currently no specific means to target the triple negative type. We have been characterizing acute and chronic phases of hypoxic response, and revealed the mechanisms to regulate transcription and metabolism under such conditions. In this project, we aim to understand the character of triple negative type breast cancers by combining the knowledge of epigenetics, chromatin conformation and metabolism (Figure). Our final goal is to identify a systemic biomarker to detect breast cancers at an early stage by combining the molecules identified in this project.
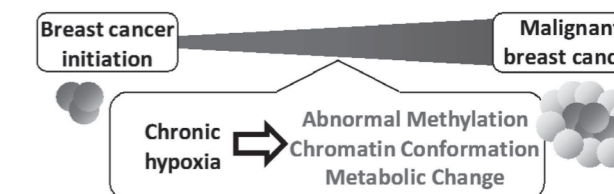

Figure. Outline of hypoxic breast cancer project

Core research project for the implementation of Precision Medicine of Head and Neck and Esophageal Squamous Cell Carcinoma

Project leader Johji Inazawa

Collaborators Takahiro Asakage

Tohru Ikeda, Masanobu Kitagawa, Tatsuhiko Tsunoda,

Toshihiro Tanaka, Hiroyuki Harada, Satoshi Miyake,

Yasuaki Nakajima, Sadakatsu Ikeda, Keiichi Morita,

Akira Takemoto, Kousuke Tanimoto, Tomoki Muramatsu

Head and neck squamous carcinoma (HNSCC), including oral SCC (OSCC), and esophageal squamous cell carcinoma (ESCC) tend to easily metastasize to lymph nodes, so that the prognosis is poor. In addition, surgical treatment against HNSCC and ESCC deteriorates the quality of life due to the resulting changes in the facial appearance and difficulties posed to meal intake. Recently, several clinical trials using immune checkpoint inhibitors (ICIs) for HNSCC and ESCC have been conducted, where it has been revealed that ICIs could improve the survival of patients with HNSCC and ESCC by reactivating the anticancer immune response. However, despite the knowledge on HNSCC and ESCC, specific medicines against both cancers have not been developed so far. In this project, our aim is to establish precision medicine for HNSCC and ESCC through the analysis of omics data using information of genomics, epigenomics, transcriptomics, proteomics, metabolomics and phenomics.

Research outcome

1) Status of the collection of HNSCC and ESCC clinical samples

We have constructed a network for the collection and analysis of clinical samples through a cooperation between the Bioresource Research Center (BRC) and the medical and dental hospitals of TMDU. Over 900 clinical samples have been collected as of Sep. 2020 (HNSCC: 272 samples, ESCC: 276 samples, OSCC: 395 samples). In addition, we have performed DNA sequence analysis with clinical samples of several cancer types including HNSCC (26 samples) and ESCC (17 samples) to understand the relationship between gene mutations and the various cancer states.

2) Development of microRNA-based cancer therapeutics

To investigate novel Tumor suppressive-miRs (TS-miRs) for the development of miRNA-based cancer therapeutics, we examined a total of 2,565 miRs, which covered ~96% of the registered human miRs, on the basis of their growth-inhibitory effects in many types of cancer cell lines including ESCC and OSCC. As a result, we identified *miR-1293* as a novel TS-miR and revealed the mechanisms by which *miR-1293* reduced cell growth. *miR-1293* directly suppressed BRD4 and several DNA repair genes, inducing apoptosis in cancer cells. In addition, administration of *miR-1293* suppressed *in vivo* tumor cell growth. These results indicated that *miR-1293* might be a potential candidate for the development of miRNA-based cancer therapeutics (Takagawa et al Mol Ther. 2020).

3) Exploring novel anticancer drugs based on drug repurposing

Drug repurposing (DR) is a widely used strategy that seeks to identify new medical indication for drugs that are already approved for the treatment of an original disease. The merit of DR involves the use of lower risk compounds that offer lower costs and shorter timelines in their development. Thus, we aimed to find novel anticancer drugs through a functional cell-based screening of an FDA-approved drug library against OSCC and ESCC. Pitavastatin, an HMGCR inhibitor, emerged as an anticancer drug that inhibits tumor growth by downregulating AKT and ERK signals in OSCC and ESCC cells. One of the mechanisms by which pitavastatin inhibits cell growth might be the suppression of MET signaling through immature MET due to dysfunction of the Golgi apparatus. Moreover, the sensitivity of tumor growth to pitavastatin might be correlated with *GGPS1* expression levels. *In vivo* therapeutic models revealed that the combination of pitavastatin with capmatinib, a MET-specific inhibitor, dramatically reduced tumor growth. Our findings suggest that *GGPS1* expression could be a biomarker in cancer therapy with pitavastatin, and the combination of pitavastatin with capmatinib might be a promising therapeutic strategy in OSCC and ESCC (Xu B et al. Mol Cancer Res. 2020).

Publications

1. Takagawa Y, Gen Y, Muramatsu T, Tanimoto K, Inoue J, Harada H, Inazawa J: miR-1293, a candidate for miRNA-based cancer therapeutics, simultaneously targets BRD4 and the DNA repair pathway. Mol Ther. 2020 28:1494-1505.
2. Xu B, Muramatsu T, Inazawa J: Suppression of MET signaling mediated by pitavastatin and capmatinib inhibits oral and esophageal cancer cell growth. Mol. Cancer Res. 2020 Online ahead of print.

DOHaD research towards preventive and preemptive approach against chronic intractable disease

Project Leader Noriko Sato

Collaborators Naoyuki Miyasaka

Tomonori Ishikawa

Ayako Fudono

Sumio Sugano

Chihiro Imai

Summary

Multiple lines of evidence from epidemiological observations have implicated that the quality of fetal development is linked to risks of non-communicable diseases later in life. Developmental Origin of Health and Disease (DOHaD) was conceptualized, which means that the developing conditions *in utero* or the early phase of life will modify the long-lasting bodily function and physiology. In this context, the problem of an exceptionally high percentage of low-weight-births in a super-aged society, Japan, has been raised as a serious concern. To implement preemptive medicine from the early stage of life, it is important to elucidate how the environment interacts with the fetal genome and modulates its phenotype. We are currently continuing our Birth Cohort – Gene and ENvironment Interaction Study of TMDU (BC-GENIST). Furthermore, we have prepared to initiate the collaboration with the ToMMo's Birth and Three-Generation Cohort and the Hamamatsu Birth Cohort groups, to expand DOHaD research.
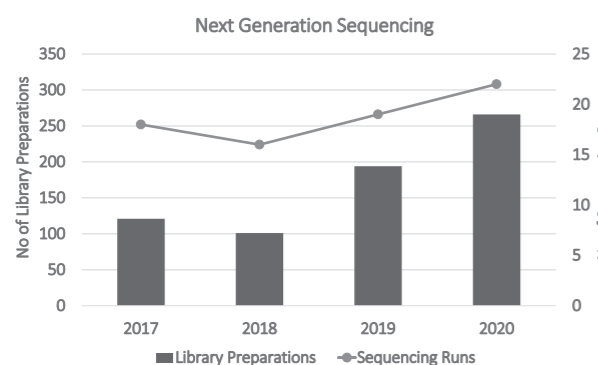
Research Project

One of the causes of lowering birth weight in Japan may be the underweight of pregnant women. Multiple studies have reported that the energy intake of pre-pregnant and pregnant women is mostly deficient, and the same trend was consistently observed in BC-GENIST. However, energy intake is relatively susceptible to measurement error in dietary surveys. We evaluated the quality of the diet by focusing on qualifying and disqualifying nutrients, and scoring the conformity with Dietary Reference Intakes (DRIs) for Japanese, 2015. The results showed that pregnant women with the higher scores, indicating the better nutrition status, tended to have lower pre-pregnancy weight, pre-pregnancy BMI, and gestational weight gain. It suggested the importance of considering diet quality upon the nutritional management for pregnant women beyond BMI-based management. Furthermore, iron and/or vitamin D deficiencies are frequently observed in women in Japan, and there is concern about the effects of these deficiencies on fetal growth. We are currently investigating the effect of maternal iron deficiency on the DNA methylation status of the chromatin regions modulated by heme and BACH1/2 transcription factors. Also, we are collaborating with Dr. Masako Suzuki of Albert Einstein College of Medicine in the U.S. to study the effect of maternal vitamin D deficiency on the immune cell proportions of offspring.

Project for Hereditary Osteolysis

Project Leader Yoichi Ezura

To develop an effective management strategy for the treatment of hereditary osteolysis, we sought a therapeutic agent that would improve the symptoms of mice that specifically lack profilin 1 (Pfn1) in osteoclasts. This model mouse genetically represent the symptoms of the novel type of Paget's disease of bone (PDB) with early onset and malignancy: *PFN1* mutation was first identified in a large pedigree holding 11 affected individuals last year (2020). In our project, we continued to evaluate the efficacy of available therapeutics on this model mouse and derivative osteoclasts in culture, using alendronate and an inhibitor of Arp2/3 (CK-666). We also started genetic analysis for etiologically unknown osteolytic disorder "Gorham disease" by collaboration with professor Inazawa at department of cytogenetics in our institute.

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. We have cooperated with Research Core of the University.
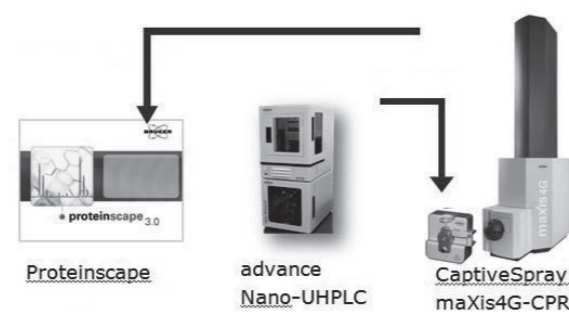
Followings are the achievements in 2020.

1. Sequencing analyses

A total of 17,432 samples from 1,914 researchers were sequenced in the year of 2020. Among them 9,615 (55%) samples were requested by researchers outside the medical Research Institute (see below). Analysis by using next generation sequencing equipment (Ion PGM and Ion S5) has been started in 2013 and 22 runs were done in the year of 2020. Library preparation service for next generation sequencing has been started in 2015 and 266 samples were done in the year of 2020.

2. Equipment under the management of the Genome Laboratory.

DNA sequencer (ABI3130xl) × 2, Next generation sequencer (Ion PGM, Ion S5), PCR machine (ABI7900) × 5, Flow Cytometer, Luminescent Plate Reader, Ice Machine, Centrifuge, Ion One Touch system, DNA/RNA shearing system, and others.

Next Generation Sequencing

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 Installations written below.

For proteome analysis, we have LC-MSMS systems and a MicrocalorimetryTC200, Eppendorf InjectMan NI2, Leica M165FC in this laboratory. We can accept the consignment analysis of proteins with the mass spectrometry by request of researchers in this university. In addition, we can provide technical advices on cytometry and proteome researchers who wish to start their own research.

We belong to RCC(Research Core Center) and Nanken-Kyoten inTMDU.

Proteinscape advance Nano-UHPLC CaptiveSpray maXis4G-CPR

maxis-4G-CPRsystem Bruker Japan

Laboratory of Genome Editing for Biomedical Research

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 this 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.

1day p.c

3days p.c

Chimera mice 10 days

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-325, HM-335E (Microm)
・Vibrating microtome … PRO7 (D.S.K.)

・Automated Tissue Processor
　　　　　… RH-12DM (Sakura Finetek)
　　　　　Excelsior ES (Thermo Fisher Scientific)
・Tissue-embedding-station
　　　　　… Histostar (Thermo Scientific)
・Real-time PCR … 7500、7900HT (Applied Biosystems)
・Laser Capture Microdissection … LMD7000 (Leica)
・Stereo microscope … SZX-16 (Olympus)

<<seminars and demonstrations>>

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 held on the following dates.

Confocal laser microscope…11/10 (Carl Zeiss)

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 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 three Professors and three 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 274 in the year of 2020. We held 2 short courses for beginners to help them use the equipment.

Bioresource Laboratory

Bioresource Laboratory of Medical Research Institute provides support for researchers and postgraduates in the field of biomedical sciences, both on and off campus, in terms of bioresources.

We safely supply cultured cell lines in compliance. In this year, we received orders from the Kamakura Woman's university and provided them with a cell line. EB-virus transformed cell lines are established with B-lymphocytes from patients with intractable diseases after written informed consent from each of the patients or their parents and with approval of the Internal Review Board on ethical issues. We are constantly receiving requests for this service form our pediatrics and other research institutions. In this year, we accepted new requests from the
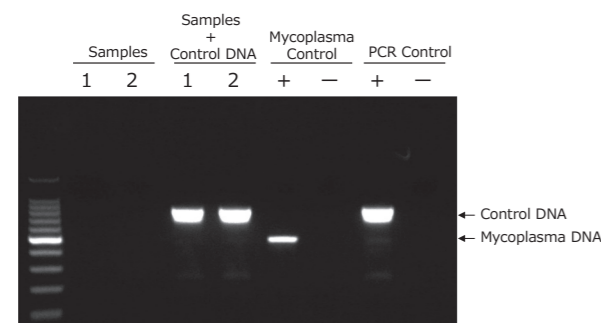
Wakayama medical university and the Hokkaido university. We also undertake with mycoplasma contamination test (see Fig.1). In this year, inspection requests decreased compared to the previous year. This is probably due to suspension of new reception for a certain period (April 1st to June 1st) and the restriction of research activities under the issuance of a state of emergency due to COVID-19. We were requested storage of many specimen biological samples from many laboratories in preservation work using a large liquid nitrogen tank (see Fig.2). Although this service is limited to the university, it is a useful service for users.

Fig. 1 Mycoplasma Test

Fig.2 Large liquid nitrogen tank G430-S (Taiyo Nippon Sanso Coporation)

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 the 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.
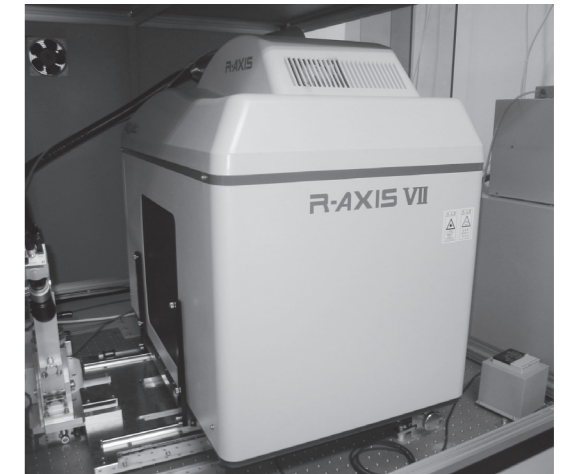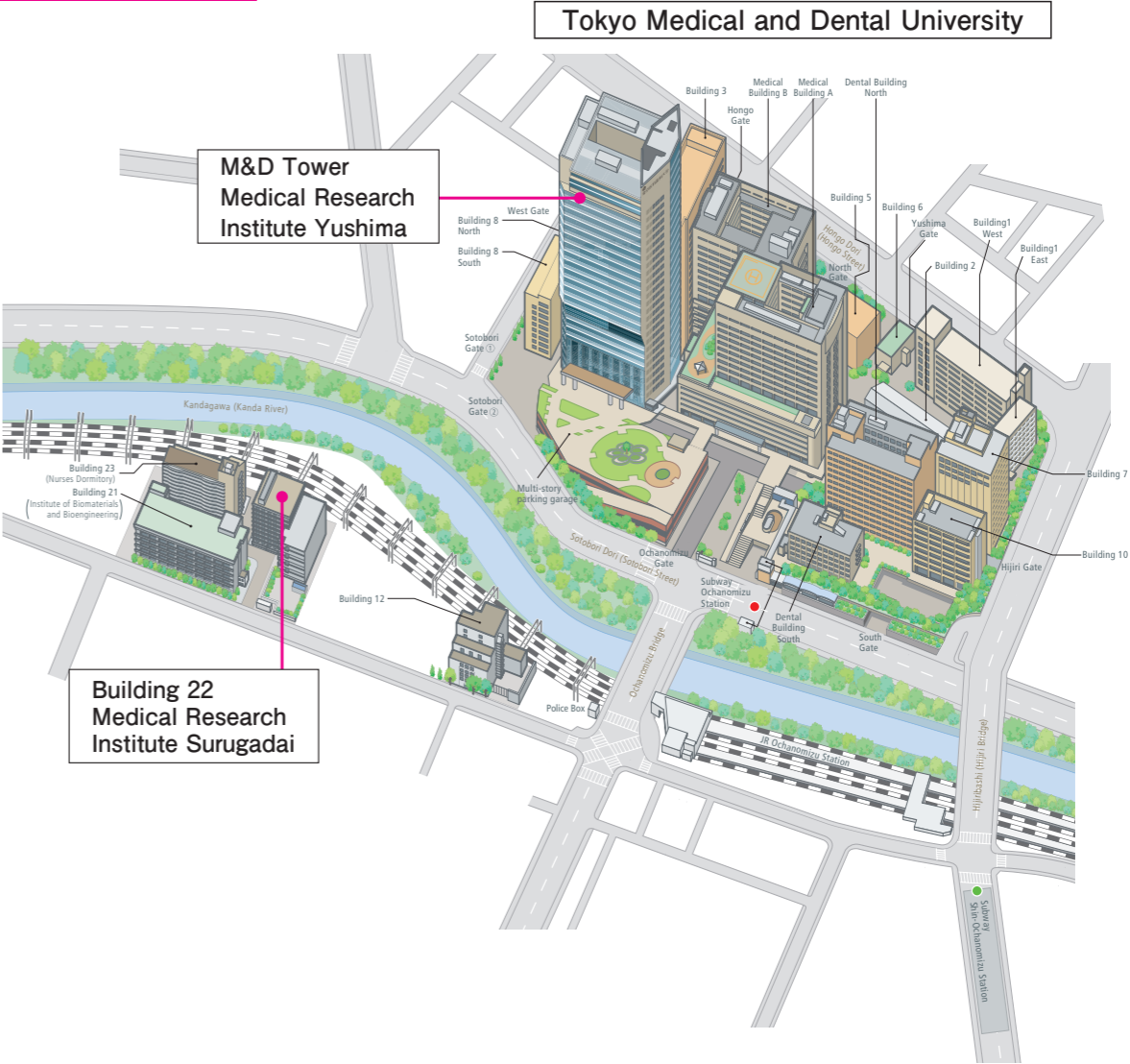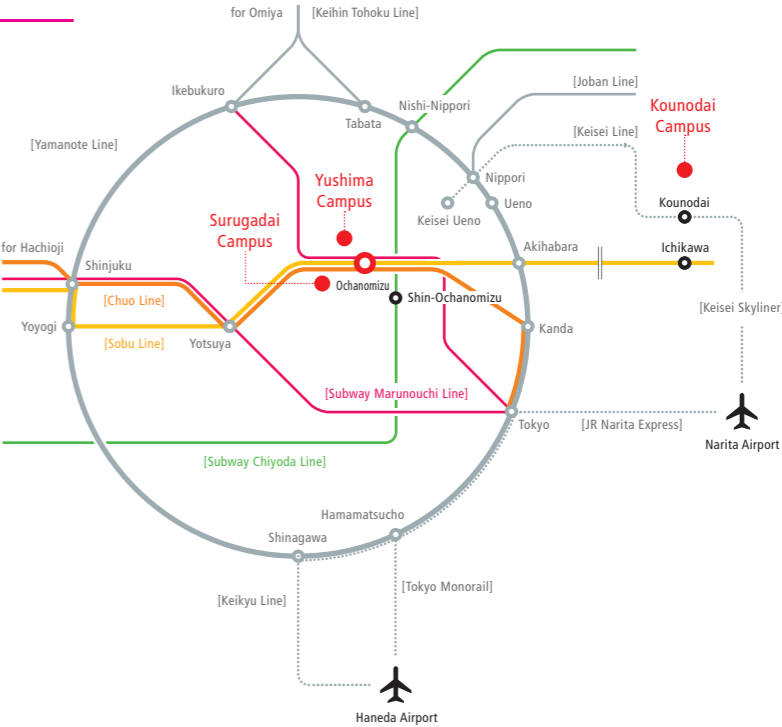

Photo: Image plate X-ray detector

Access Map

Tokyo Medical and Dental University

M&D Tower
Medical Research
Institute Yushima

Building 22
Medical Research
Institute Surugadai

Building 3
Medical Building B
Medical Building A
Dental Building North
Hongo Gate
Building 5
Building 6
Yushima Gate
Building1 West
Building1 East
West Gate
Building 8 North
Building 8 South
Building 2
Sotobori Gate ①
Sotobori Gate ②
Kandagawa (Kanda River)
Building 23 (Nurses Dormitory)
Building 21 (Institute of Biomaterials and Bioengineering)
Ochanomizu Bridge
Sotobori Dori (Sotobori Street)
Ochanomizu Gate
Multi-story parking garage
Subway Ochanomizu Station
Building 12
Building 7
Building 10
Hijiri Gate
Dental Building South
South Gate
Police Box
JR Ochanomizu Station
Subway Shin-Ochanomizu Station

Nearest Stations

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

for Omiya
[Keihin Tohoku Line]
[Joban Line]
Kounodai Campus
[Keisei Line]
Ikebukuro
Nishi-Nippori
Tabata
[Yamanote Line]
Nippori
Kounodai
Ueno
Yushima Campus
Keisei Ueno
Surugadai Campus
Akihabara
Ichikawa
for Hachioji
Shinjuku
[Chuo Line]
Ochanomizu
Shin-Ochanomizu
Yoyogi
[Sobu Line]
Yotsuya
Kanda
[Keisei Skyliner]
[Subway Marunouchi Line]
Tokyo
[JR Narita Express]
Narita Airport
[Subway Chiyoda Line]
Hamamatsucho
Shinagawa
[Keikyu Line]
[Tokyo Monorail]
Haneda Airport

ANNUAL REPORT 2021

Medical Research Institute

Tokyo Medical and Dental University

1-5-45 Yushima Bunkyo-ku Tokyo 113-8510 Japan
Tel +81-3-5803-4504