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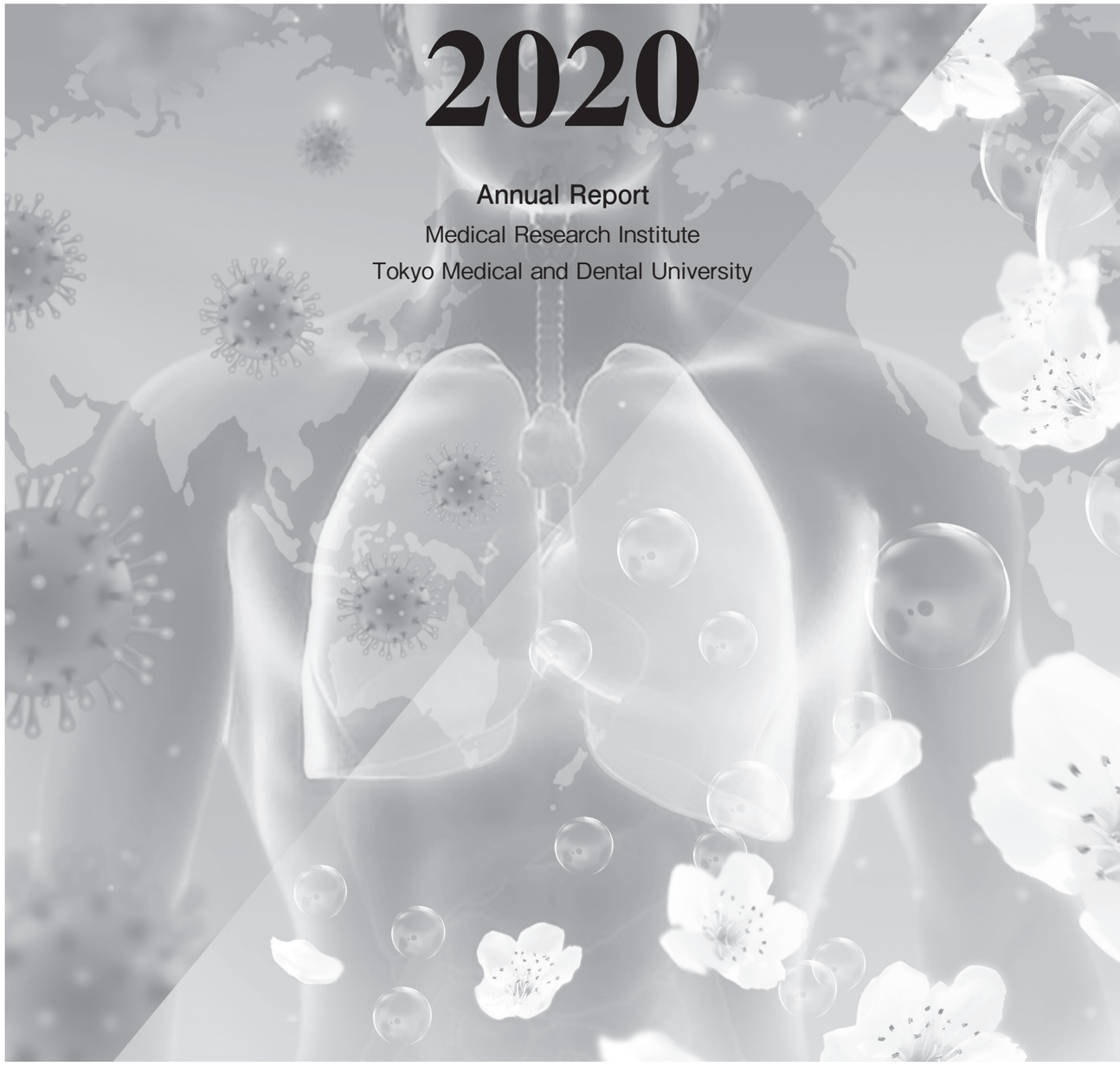
ANNUAL REPORT 2020

Medical Research Institute / Tokyo Medical and Dental University

Annual Report 2020

Tokyo Medical and Dental University



2020

Annual Report

Medical Research Institute

Tokyo Medical and Dental University

Contents

Medical Research Institute

Yushima Area

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

Medical Research Institute

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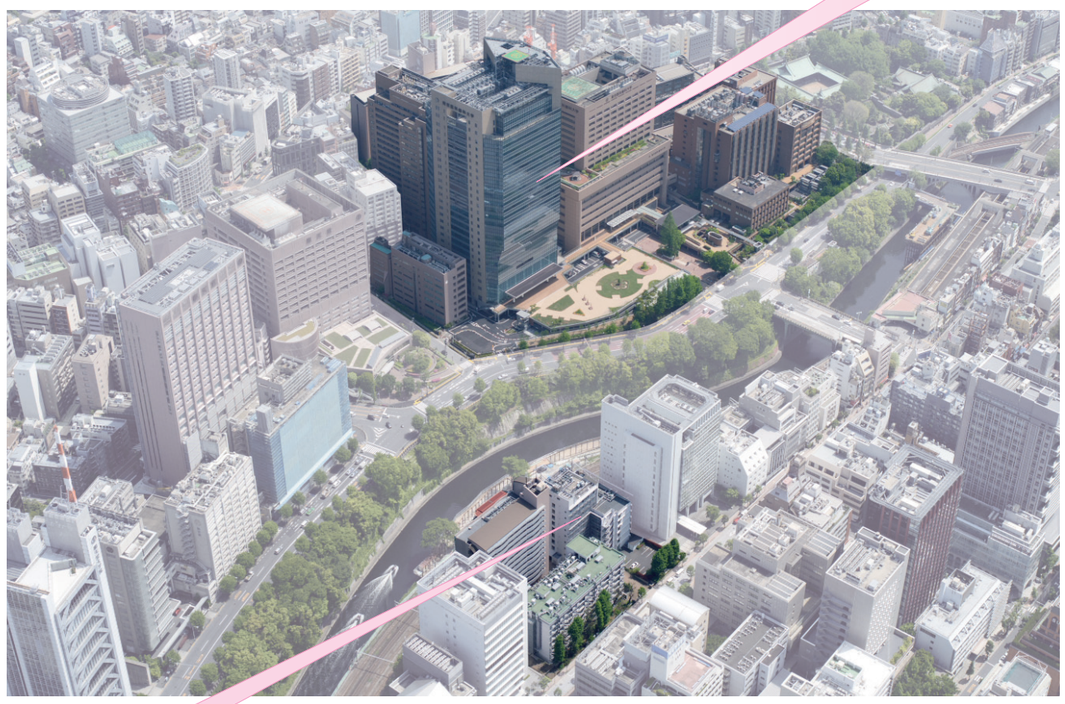


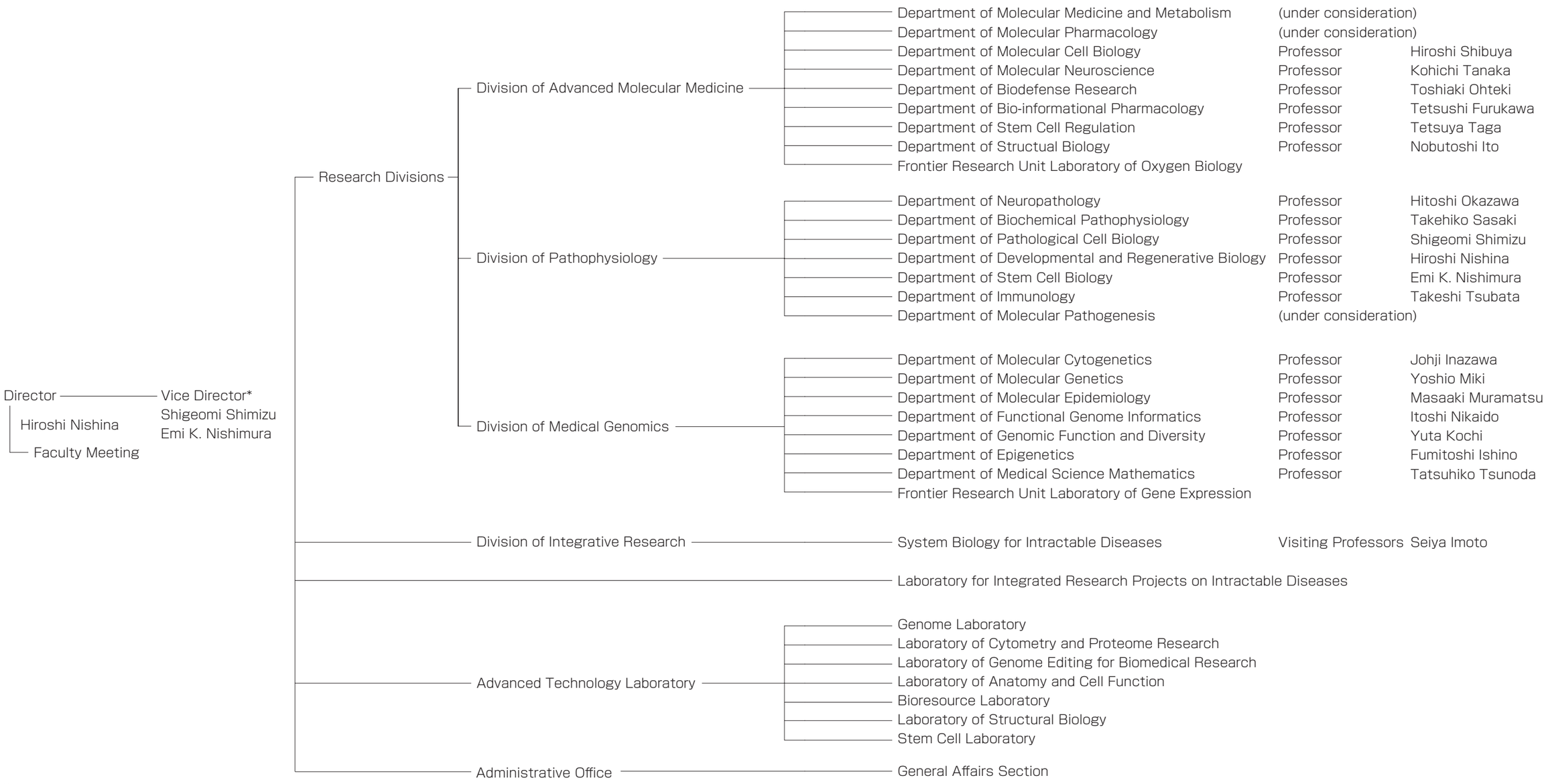
Surugadai Area

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

Medical Research Institute

Laboratory of Genome Editing for Biomedical Research

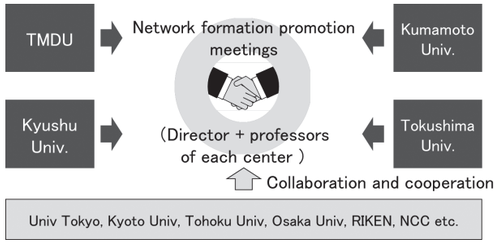
Medical Research Institute



*Provisional name

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 4th Symposium of the Inter-University Research Network for Trans-Omics Medicine

Trans-Omics for Advanced Medical Sciences (Organized by Institute of Advanced Medical Sciences, Tokushima University)

Date: November 14-15, 2019

Place: Fujii Memorial Hall, Fujii Memorial Institute of Medical Sciences, Tokushima University

Speakers from MRI, IMDU:

Yuta Kohchi (Professor, Department of Genomic Function and Diversity)

What can we learn from eQTL studies in disease genetics?

Tetsushi Furukawa (Professor, Department of Bio-informational Pharmacology)

Trans-omics approach for precision medicine of atrial fibrillation.

Jiyoung Lee (Associate Professor, Department of Epigenetics)

Generation of structural and functional heart organoids from mouse embryonic stem cells.

Yikelamu Alifu (Graduate Student D2, Department of Developmental and Regenerative Biology)

The clock components Period2, Cryptochrome1a, and Cryptochrome2a function in establishing light-dependent behavioral rhythms and/or total activity levels in zebrafish.

*** Technical seminar**

The 3rd Trans-Omics Medicine Trans-Omics Medicine Technical Seminar

Date: March 7, 2019

Place: 11st floor, M & D Tower, Lecture room 3

Lecturers:

Akihito Harada (Assistant Professor, Medical Institute of Bioregulation, Kyushu University)

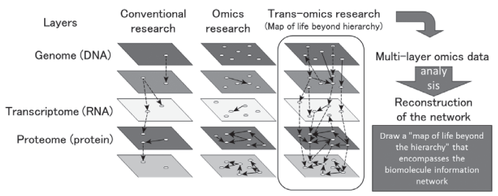
Introduction of ChIL-seq that enables chromatin modification analysis from extremely small amount of samples.

Takashi Kohda (Professor, Faculty of Life and Environmental Sciences, University of Yamanashi)

Comprehensive analysis technology for cytosine modification of genomic DNA: EnIGMA-seq

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】

- IL-8 regulates glucose metabolism including GLUT3 and GFAT expression, glucose uptake and *O*-GlcNAc modification in colon and lung cancer cells.

【Molecular Neuroscience】

- GLAST dysfunction plays important roles in the emotional and cognitive abnormalities induced by repeated PCP administration.
- Dorsal forebrain-specific GATOR1 complex knockout mice show spontaneous epileptic seizures and cerebral cortical dysplasia.

【Biodefense Research】

- Identification of novel microglia super-enhancers.
- Identification of a novel surface marker to observe bona fide hematopoietic responses under stress conditions.
- Establishment of a human tongue cancer organoid biobank.

【Bio-informational Pharmacology】

- We identified circulating biomarkers related to the onset and advancement of atrial fibrillation.
- We identified a key factor for differentiation of early mesodermal cells to cardiac mesodermal cells.
- We discovered epigenetic transcription regulation of inflammation-related genes in vascular endothelial cells.

【Stem Cell Regulation】

- Elucidation of a role of an epigenetic regulator TET1 in AGM hematopoiesis in midgestation mouse
- Elucidation and eradication of niche-constructing cancer stem cells (ncGSCs)

【Structural Biology】

- The crystal structure of nuclear receptors such as VDR and RXR with various small molecules were determined.
- A strong evidence against so-called “cistauosis” hypothesis in tau aggregation was discovered.

Department of Molecular Cell Biology

Professor	Hiroshi Shibuya
Associate Professor	Toshiyasu Goto
Assistant Professor	Atsushi Sato (~August, 2019)
Assistant Professor	Masahiro Shimizu (September, 2019~)

Cancer stem cells (CSCs), also called tumor-initiating cells, are a subset of tumor cells that exhibit self-renewal ability and generate the diverse cells that comprise the tumor. CSCs show increased quiescence and poor responses to conventional chemotherapy strategies that primarily kill proliferating cells. Therefore, CSCs are correlated with chemoresistance, invasion and relapse of cancer cells. Tumor tissues contain inflammatory cells and cancer-associated fibroblasts, which produce cytokines, chemokines and growth factors in the tumor microenvironment that influence tumor progression. Several studies have demonstrated that niches within the tumor microenvironment maintain the principle properties of CSCs. These findings suggest that the induction and maintenance of CSC properties by cytokines and chemokines may be critical for cancer development in many cancers. However, the precise mechanism underlying cytokines- and chemokines-mediated regulation of CSC properties has not been elucidated. We focus on these problems using colon and lung cancer cells.

We analyzed mechanism of CSCs development using 3D culture system called sphere formation. While 2D cultures at adherent plates can only mimic the condition of physiological tumor tissue to a limited extent, 3D cultures resemble the physiological condition of cancer cells closely compared with 2D cultures. In addition, sphere culture system selectively enriches for the growth of highly tumorigenic cancer cells showing CSC-like properties. Indeed, we revealed that CSC-like sphere-forming cells from colon and lung cancer cells showed enhanced tumorigenic activity compared with parental cells, indicating that these sphere-forming cells exhibit CSC-like properties.

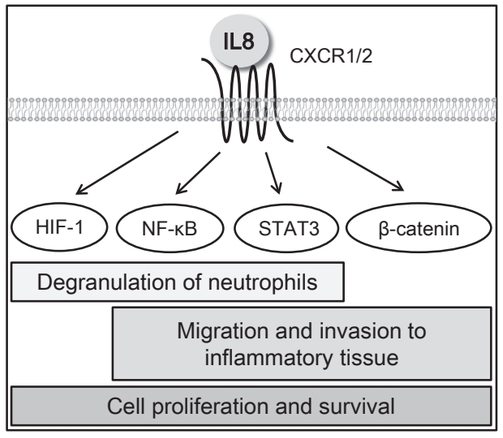


Fig.1 Cellular response in inflammatory tissue by IL-8.

1. Proinflammatory chemokine IL-8 enhances the number of CSC-like cells

We examined the expressions of cytokines and chemokines in the spheres from colon and lung cancer cells and found that the IL-8 gene was highly expressed in the spheres compared with parental adherent cells. IL-8, also known as C-X-C motif chemokine ligand 8 (CXCL8), is a chemokine that plays a pleiotropic function in the regulation of inflammatory responses through its receptor CXCR1/2 (Fig.1). The expression of IL-8 is tightly regulated and its expression level in normal tissues is very low or undetectable. In addition to its function in the inflammatory response, IL-8 also plays a critical role in cancer. IL-8 is highly expressed in many tumor types, and high serum IL-8 levels correlate with tumor size, stage and prognosis in lung, colon, breast, ovarian and prostate cancer and melanoma patients. These data suggest a role of IL-8 for development and maintenance of CSCs.

2. IL-8 regulates expression levels of GLUT3 and GFAT and enhances *O*-GlcNAcylation.

Next, we examined expression of various genes in IL-8-treated cells, and found that IL-8 promoted the mRNA and protein expression of glucose transporter GLUT3, but not GLUT1, GLUT2 and GLUT4. Consistent with GLUT3 induction, glucose uptake was enhanced by IL-8 treatment. To examine whether IL-8 activates glucose uptake and enhances glycolysis, we measured three indicators of glycolysis, intracellular ATP, lactate and pyruvate levels, in IL-8-treated cells. Interestingly, IL-8 stimulation had no effect on these glycolysis indicators both in low and high glucose conditions. These results suggest that although IL-8 induces glucose uptake by enhancing GLUT3 expression, additionally uptake glucose is not mainly utilized for glycolysis to product ATP for energy.

GlcNAcylation is the post-translational modification of N-acetylglucosamine (also known as GlcNAc) to serine or threonine residues of proteins that contributes to stability and activity of modified proteins. The GlcNAc is produced by the hexosamine biosynthetic pathway (HBP) which is derived from the glycolysis intermediate fructose 6-phosphate. We speculated that IL-8 enhanced *O*-GlcNAcylation through induction of glucose uptake. Indeed, we found that IL-8 stimulation increased the levels of proteins modified by *O*-GlcNAcylation and the expression of GFAT, which is rate-limiting enzyme of HBP. In addition, knockdown of IL-8 decreased levels of *O*-GlcNAc-modified proteins and ectopic GLUT3 expression rescued this reduction. Furthermore, IL-8-induced enhancement of *O*-GlcNAcylation was attenuated by GLUT3 and GFAT knockdown. These results suggest that IL-8 regulates *O*-GlcNAcylation via GLUT3 and GFAT in colon and lung cancer cells.

3. IL-8-induced *O*-GlcNAcylation is essential for maintenance of CSC properties.

Recent studies showed that elevated *O*-GlcNAc modification is closely related with metastasis and recurrence of various cancers, including colon and lung cancers. Therefore, we examined whether IL-8 regulates CSC properties through enhanced GLUT3 and GFAT expression and *O*-GlcNAcylation, and showed that IL-8-induced *O*-GlcNAc modification and sphere formation were suppressed by knockdown of GLUT3 or GFAT. Moreover, while knockdown of IL-8 resulted in reduced sphere formation and tumor volume compared with control, ectopic GLUT3 expression rescued IL-8 knockdown-mediated suppression of sphere and tumor development. These results suggest that IL-8-mediated enhancement of *O*-GlcNAc modification through GLUT3 and GFAT is essential for CSCs properties including tumor-forming activity.

In conclusion, we show that IL-8 regulates glucose metabolism including GLUT3 and GFAT expression, glucose uptake and *O*-GlcNAc modification in colon and lung cancer cells. We also demonstrate that IL-8-induced glucose metabolism is a key event for driving CSC-like characteristics of these cells (Fig.2).

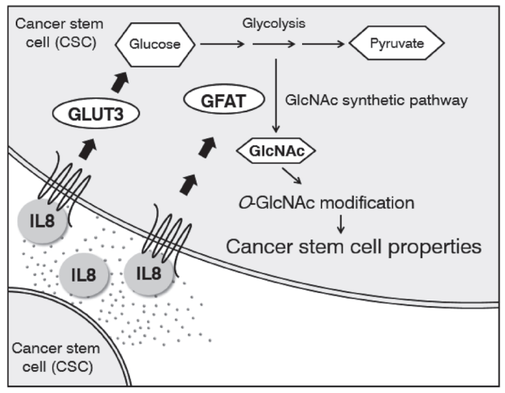


Fig.2 Mechanism of CSC properties regulated by IL-8

Publications

Shimizu M and Tanaka N. IL-8-induced *O*-GlcNAc modification via GLUT3 and GFAT regulates cancer stem cell-like properties in colon and lung cancer cells. ***Oncogene*** 38, 1520–33 (2019).

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.

Human genetic studies have suggested that de novo mutations in GLAST (EAAT1) is linked to schizophrenia. Consistent with this, GLAST null mice show phenotypes relevant to positive, negative and executive/cognitive symptoms of schizophrenia, including novelty-induced locomotor hyperactivity, abnormal social behavior characterized by reduced initiation of social interactions, poor nesting and impaired pairwise visual discrimination learning. Repeated phencyclidine (PCP) administration induces several schizophrenia-like psychobehavioral abnormalities and decreased extracellular glutamate levels, which are associated with increased expression of GLAST in the prefrontal cortex of mice. In this study, we investigated the functional roles of GLAST in the schizophrenia-like psychobehavioral abnormalities induced by repeated PCP administration using GLAST heterozygous ($GLAST^{+/-}$) mice. PCP-administered GLAST wild-type (+/+) mice showed enhancement of immobility in a forced swimming test, impairments of visual recognition memory in a novel object recognition test, decrease in high potassium (K^+)-induced extracellular glutamate release, and overexpression of GLAST and S100 proteins in the PFC, compared to saline-administered $GLAST^{+/+}$ mice. Such behavioral and neurochemical abnormalities were not observed in PCP-administered $GLAST^{+/-}$ mice. These results clearly suggest that overexpression of GLAST and glial activation play important roles in the development of emotional and cognitive abnormalities in PCP-administered $GLAST^{+/+}$mice. It is therefore necessary to strictly regulate the expression of GLAST to maintain normal brain function. Studies targeting GLAST may lead to the development of medications for emotional (negative symptoms) and cognitive impairments in schizophrenia.

Fig1. Glutamate transporter dysfunction leads to neuropsychiatric diseases

2. Elucidation of the effect of abnormalities in the GATOR1 complex on focal epilepsy

Epilepsy is characterized by recurrent seizures resulting from excessive neuronal discharge and presents a wide variety of clinical symptoms. Although epilepsy is a frequent neurological disorder that occurs in about 1% of the population, there is often no fundamental cure for it, forcing it to rely on symptomatic treatment with prolonged use of antiepileptic drugs. In addition, seizures in about 30% of patients fail to respond to the drugs. Therefore, the development of new treatment and prevention methods is urgent.

"Focal epilepsy", in which the site of abnormal neuronal firing is limited to a part of the cerebral hemisphere, accounts for half of adult epilepsy. Gap activity toward Rags 1 (GATOR1) complex abnormality is involved in about 10% of the onset of focal epilepsy. The GATOR1 complex is a complex composed of DEP domain-containing protein 5 (DEPDC5) and Nitrogen permease regulator 2 / 3-like protein (NPRL2, NPRL3). It suppresses the mechanical target of rapamycin complex1 (mTORC1) pathway that controls cell growth and proliferation (Fig.2). However, the function of the GATOR1 complex in the nervous system has not been clarified. We aim to elucidate the function of the GATOR1 complex and the role on epileptogenesis using mouse models.

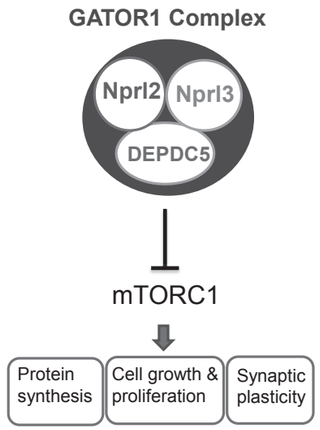


Fig2. GATOR1 complex and mTORC1 pathway

Because conventional knockout (KO) of DEPDC5, NPRL2, and NPRL3 in mice results in embryonic lethal, it was impossible to analyze their functions in adults. In this year, we focused on the cerebral cortex where most seizure focus exist, and generated the conditional knockout (cKO) mice of each gene. Phenotypic analysis revealed that each cKO mouse exhibited spontaneous epileptic seizures and cerebral cortical dysplasia similarly to patients, indicating that they are useful as epilepsy model mice (Fig.3). Further analysis of these mice will promote our research on elucidation of the onset mechanism.

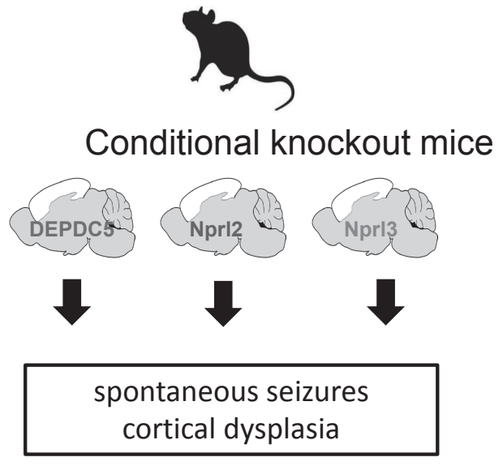


Fig3. Each cKO mouse exhibits spontaneous epileptic seizures and cortical dysplasia

Publications

[Original papers]

1. Uchida M, Hida H, Mori K, Yoshimi A, Kitagaki S, Yamada K, Hiraoka Y, Aida T, Tanaka K, Ozaki N, Noda Y. Functional roles of the glial glutamate transporter (GLAST) in emotional and cognitive abnormalities of mice after repeated phencyclidine administration. Eur *Neuropsychopharmacol* 29. 914-924, 2019.
2. Yamada T, Takechi M, Yokoyama N, Hiraoka Y, Ishikubo H, Usami T, Furutera T, Taga Y, Hirate Y, Kanai-Azuma M, Yoda T, Ogawa-Goto K, Iseki S. Heterozygous mutation of the splicing factor Sf3b4 affects development of the axial skeleton and forebrain in mouse. *Dev Dyn* doi: 10.1002/dvdy.148. 2020.
3. Hirayama T, Hiraoka Y, Kitamura E, Miyazaki S, Horie K, Fukuda T, Hidema S, Koike M, Itakura A, Takeda S, Nishimori K. Oxytocin induced labor causes region and sex-specific transient oligodendrocyte cell death in neonatal mouse brain. *J Obstet Gynaecol* 46. 66-78, 2020.
4. Mulati M, Kobayashi Y, Takahashi A, Numata H, Saito M, Hiraoka Y, Ochi H, Sato S, Ezura Y, Yuasa M, Hirai T, Yoshii T, **Okawa** A, Inose H. The long noncoding RNA Crnde regulates osteoblast proliferation through the Wnt/ β-catenin signaling pathway in mice. *Bone* 130. 115076, 2020.

Department of Biodefense Research

Professor	Toshiaki Ohteki, DDS, Ph.D.
Junior Associate Professor	Taku Sato, Ph.D.
Assistant Professor	Masashi Kanayama, Ph.D.
Adjunct Lecturer	Nobuyuki Onai, Ph.D.
Restart Postdoctral Fellowship (JSPS)	Mihoko Kajita, Ph.D.
Research Technician	Shoko Kuroda
Research Technician	Kisho Shiseki
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 mononuclear phagocytes

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

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

Based on these achievements in mouse, we have been trying to identify human progenitors of mononuclear phagocytes, and most recently succeeded to identify human cMoP (***Immunity*** 2017; ***Int Immunol*** 2018). Human cMoP gives rise to only monocytes but not other hematopoietic cells including DCs. Given that monocytes and monocyte-derived macrophages cause a variety of inflammatory disorders, including metabolic syndromes and tumor development, our studies shed light on possible therapeutic applications for infectious diseases, cancers and autoimmune diseases. Collaborations with pharmaceutical company and Department of Hematology of TMDU toward the development of therapeutic agents targeting cMoP and monocyte lineage and with Department of Pediatrics of TMDU for the pathology clarification of congenital pulmonary alveolar proteinosis (PAP) are currently in progress.

2) Mechanism of brain function impairment by spatiotemporal transformation of microglial enhancer

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.

In this study, using a novel technology that can detect the active enhancer region and its activity with high sensitivity, we will identify the super enhancers (hereafter, SEs) responsible for the microglial transformation during life-stage progression, and elucidate the entire process of transformation dynamics. As SEs are activated in a cell-type specific manner, one can expect that it will lead to the development of novel technology to specifically control the age-related functional alteration of microglia. To date, 36,320 new microglial enhancers including 937 regions that become different with age have been identified, and the coding regions regulated by these enhancers are being analyzed by Hi-C technology (**Fig. 1**, unpublished).

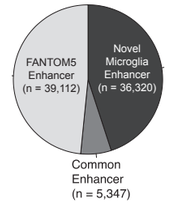


Fig.1 Identification of novel microglia enhancers by NET-CAGE analysis

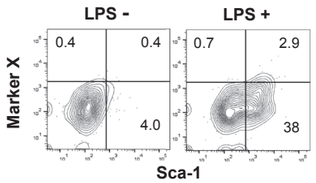


Fig.2 HSPC marker X that does not change during stress hematopoiesis
LPS stimulated hematopoietic progenitor increase Sca-1 expression but not marker X.

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(s) 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 marker with less fluctuation during emergency myelopoiesis (**Fig. 2**, in revision). Using this unique marker, we will elucidate the mechanism of emergency myelopoiesis.

2. Research on tissue stem cells

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

We found that type I IFNs induce proliferation and exhaustion in hematopoietic stem cells (HSCs), and that interferon regulatory factor-2 (IRF2), a transcriptional suppressor of type I IFN signaling, preserves the self-renewal and multi-lineage differentiation capacity of HSCs (***Nat Med*** 2009; ***Blood*** 2013). Based on these achievements, we have further found that physiological levels of type I IFN signaling also affect other tissue stem cells (in revision).

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, the balance of YAP/Wnt signal is important for the epithelial regeneration ability of the origin cells, and one of the tetraspanin family members is a useful marker to identify the fraction including the cell of origin (in revision).

2) Establishment of biobank for human tongue cancer

Oral cancer has an increasing trend of 300,000 new cases per year worldwide. Two-thirds of them are tongue cancers, 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, e.g. buccal mucosa cancer, oral floor cancer, LN-metastasized TC, and esophageal cancer. These TC organoid lines can reproduce the original TC tissues and are useful for accurate risk grading and predicting prognosis at early stages of TC, which is difficult to diagnose clinically. In addition, we identified new therapeutic target molecules (submitted). In the future, we aim to develop fundamental technologies that lead to personalized treatment.

Publications

1. Wang Z, Adachi S, Kong L, Watanabe D, Nakanishi Y, Ohteki T, Hoshi N, Kodama Y. Role of eosinophils in a murine model of inflammatory bowel disease. **Biochem Biophys Res Commun.** 2019 Mar 26;511(1):99-104. DOI: 10.1016/j.bbrc.2019.02.056

2. Hiroyuki Tezuka, Toshiaki Ohteki, Regulation of IgA Production by Intestinal Dendritic Cells and Related Cells. **Front Immunol.** 2019 August 13 ; 10 : 1891 doi.org/10.3389/fimmu.2019.01891

3. Adachi T, Yoshikawa S, Tezuka H, Tsuji NM, Ohteki T, Karasuyama H, Kumazawa T.Propolis induces Ca2+ signaling in immune cells. **Biosci Microbiota Food Health.** 2019;38(4):141-149. doi: 10.12938/bmfh.19-011. Epub 2019 Aug 24.

Personnel Changes
Kajita M, Restart Postdoctral Fellowship (JSPS).

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.
Post-doctoral Fellow	Masahiro Yamazoe, M.D., Ph.D.
	Yoshitake Higashijima, 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. Heart conduction system specific gene transduction utilizing in vivo genome editing

His-Purkinje system is the conduction system to propagate the electrical excitation through the whole heart with appropriate timing, which is essential to obtain the blood flow out of it efficiently. Recently, His-Purkinje system has been thought to be involved in the pathogenesis of many cardiac diseases, such as lethal tachyarrhythmia, bradycardia, heart failure and so on, and to be an attractive therapeutic target. Although device based approach to His-Purkinje system has been well developed, non-invasive His-Purkinje cell specific approach has not developed so far. Based on combination of cardiotropic AAV9 vector, cardiac specific promoters, and CRISPR/Cas9, we developed novel gene transduction method specifically to His-Purkinje cells. This technique is expected to be applicable non-invasive biological pacemaker generation in situ.

2. Functional analysis of novel single nucleotide polymorphism identified in exome-wide association study targeting atrial fibrillation

Recently we conducted exome-wide association study (ExWAS) targeting atrial fibrillation (AF), and identified a novel rare single nucleotide polymorphism (SNP) associated with AF. This gene was known as a transcriptional factor expressed in nervous tissue. We aimed to investigate the function of this gene on the pathogenesis of AF, focusing on an autonomic nervous system. In vitro transfection experiment revealed that both of wild type (WT) and mutant gene increased tyrosine hydroxylase (TH) expression than control. In addition, TH level in overexpression of mutant gene was significantly higher than that in WT. In next we generated the susceptibility SNP knock-in (KI) mice of this gene using CRISPR/Cas9. Heart rate variability analysis showed that KI had significantly increased sympathetic activity component than WT. Burst stimulation to left atrium evoked AF only in KI mouse (55.6%), but not in WT (0%). Collectively, novel SNP identified in ExWAS may increase the AF vulnerability via enhancing sympathetic nervous tone.

3. Understanding the mechanisms of heart induction and its compartment:

Understanding the defined factors during heart development：to address synergistic functions in genetic/physiological levels, we are focusing on *Tbx5* associate factors as model genes that mutations cause/enhance heart diseases.

Understanding the epigenetic factors regulating the congenital heart defects in human：we are focusing on the chromatin/histone modification factors. We succeeded *in utero KO* mice of lncRNAs with hypomorphic left ventricle.

Understanding the heart cell fates during development/regeneration：using microdissection system (Leica) from developing mouse models and iPS models, we generated the bioinformatic method after single-cell analysis (Fuidigm). Finally, we identified key factors specifying the heart cell and prevent vessel formation from the mesodermal cells during development.

4. The histone demethylases KDM7A and UTX regulate early inflammatory response via chromatin reorganization during atherogenesis

H3K9me2 and H3K27me3 are generally linked to gene repression. However, the functions of repressive histone methylation dynamics during inflammatory responses remain unknown. Here, we found that TNF-α rapidly induces the co-occupancy of lysine demethylases 7A (KDM7A) and 6A (UTX) with NF-κB recruited elements in human endothelial cells. KDM7A and UTX demethylate H3K9me2 and H3K27me3, respectively, and both are required for induction of NF-κB-dependent inflammatory genes. Chromosome conformation capture-derived methods including Hi-C and ChIA-PET revealed increased interactions between TNF-α-induced super enhancers at NF-κB-relevant loci, coinciding with KDM7A- and UTX-recruitment. Pharmacological inhibition of KDM7A and UTX significantly reduced leukocyte adhesive events in mice. Collectively, these findings suggest that rapid erasure of repressive histone marks by KDM7A and UTX is essential for NF-κB-dependent regulation of genes that control inflammatory responses of endothelial cells.

Highlight

Precision medicine of atrial fibrillation (AF) (Figure)

AF is the most frequent arrhythmia and referred as “cardiac pandemic in 21th century” with about one million patients. About 5% of those with AF develop stroke per year and about one out of 5 bed-redden individuals is caused by AF. Development of new anticoagulants improved the incidence of stroke per year to less than 1%. However, AF starts as paroxysmal AF, and nearly half of patients are asymptomatic, and thus cannot be diagnosed, referred as latent AF. The development of stroke in latent AF patients, called embolic stroke of unknown source (ESUS) has emerged as unmet-need. We have started clinical study to identify latent AF using AI analysis of 4 different information, genome, life-style, biomarker, and ECG (https://www.amed.go.jp/koubo/02/01/0201C_00059.html).

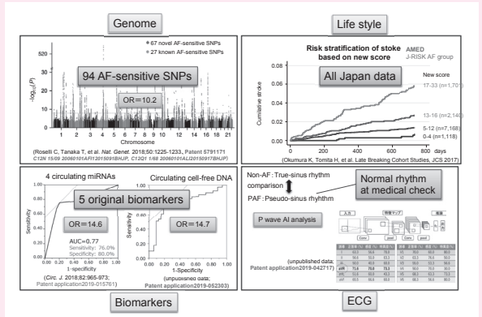


Figure. Four different information correlated with AF
Upper left:genome, upper right:life style, lower left:biomarkers, lower right ECG

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8. **Higashijima Y**, Nagai N, Kitazawa T, Kawamura Y, Kurihara H, Wada Y, Kanki Y. Lysine demethylase 7a regulates the anterior-posterior development in mouse by modulating the transcription of Hox gene cluster. ***bioRxiv*** **2019, doi: https://doi.org/10.1101/707125.**

Department of Stem Cell Regulation

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Assistant Professor	Kouichi TABU
Adjunct Lecturer	Ryoichiro KAGEYAMA, Taichi KASHIWAGI
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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 2018 are mainly on two subjects: 1. Characterization of fetal hematopoiesis, and 2. Characterization of cancer stem cells and their niche.

Research Projects

1. The contribution of TET1 to the maintenance of the hematopoietic capacity in hematopoietic stem cell-containing clusters in the dorsal aorta in midgestation mouse embryo

In midgestation mouse embryo, the dorsal aorta in aorta-gonad-mesonephros (AGM) region has hematopoietic stem cell-containing intra-aortic hematopoietic clusters (IAHCs). We previously reported that forced expression of a transcription factor *Sox17*, which is expressed in IAHCs, into CD45^{low}c-Kit^{high} AGM cells, which are one component of IAHCs, maintains the formation of hematopoietic cell clusters with hematopoietic activity. TET1 (ten-eleven translocation methylcytosine dioxygenase 1), which is involved in demethylation of 5-methylcytosine, is revealed to be highly expressed in Sox17-transduced cells by semi-quantitative RT-PCR. Therefore, we analyzed the role of TET1 in hematopoietic ability of IAHCs in the AGM region. The TET1 expression was observed in cluster cells and endothelial cells of the dorsal aorta by in situ hybridization and immunohistochemistry. Moreover, in the AGM region, we observed a low expression level of TET1 in hematopoietic cells and high expression levels of TET1 in endothelial cells and CD45^{low}c-Kit^{high} cells, the latter of which have high hematopoietic ability. To examine the role of TET1 on the maintenance of hematopoietic ability, shRNA-mediated knockdown of TET1 was performed in Sox17-transduced cells. TET1 knockdown led to a decrease in the ability to form multi-lineage hematopoietic colonies in methylcellulose media. Furthermore, we imply from luciferase assays that transient expression of Sox17 activated the TET1 promoter, in which Sox17-binding sequences exist. These results raise the possibility that TET1 contributes to maintaining the undifferentiated state in Sox17-transduced cells (Figure 1).

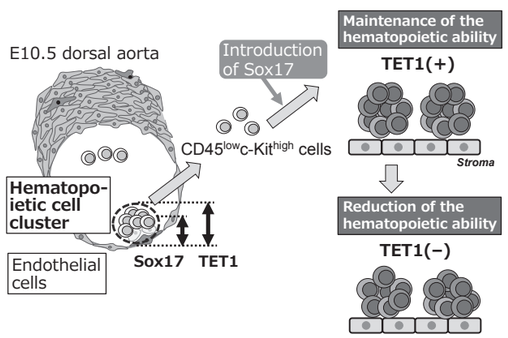

Figure 1: Model of the role of Sox17 and TET1 in hematopoietic ability of intra-aortic hematopoietic cell clusters

2. Elucidation and eradication of niche-constructing cancer stem cells (ncGSCs)

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 multiform, 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. Recently we have reported that glioma CSCs (GSCs) have a self-expanding strategy that constructs niche by facilitating the development of CD204(+) and CD11c(+) tumor-associated macrophages (TAMs) through secreting a cytokine GM-CSF (Figure 2). In this year, we aimed to elucidate such niche-constructing GSCs (ncGSCs) as a therapeutic target and establish new therapeutic strategies against them. In silico analysis using CIBERSORT and TCGA transcriptome data of 528 GBM specimens, we initially explored GSC markers correlating with the proportions of individual immune cell types in tumors. Among GSC-specific genes, the monocyte chemoattractant gene CCL2 was identified as a best candidate of ncGSC marker, whose expression is correlated with not only the recruitment of monocytes/macrophages but also poor prognosis of GBM patients. In the established reporter cells in which fluorescent gene is co-transcribed with endogenous CCL2 gene, CCL2(high) cells were found to account for 10 to 70 % of GSCs. Importantly, CCL2(high) cells were dramatically enriched by the treatment of conventionally used small-molecule drugs TMZ and ACNU, suggesting that CCL2 is a potential marker for chemoresistant GSCs leading to glioma recurrence. Aiming to extracellularly regulate/target ncCSCs, we next screened for synthetic large-molecules, i.e. polymers as cell-scaffolds for CCL2(high) cells and identified several hit polymers that support or arrest their expansion. Further investigation using these hit polymers could provide clues to develop new therapieutics to eradicate GBMs.

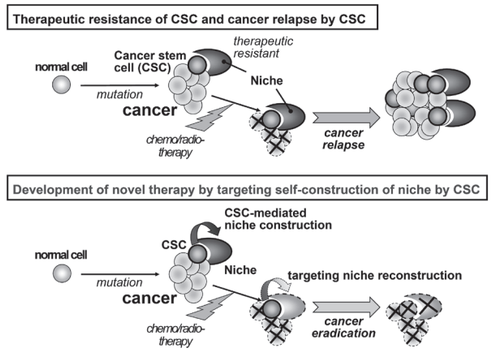

Figure 2: The concept of CSC-mediated niche construction and cancer eradication

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. Crystal structure of PDZ domain of intracellular signaling protein

Dishevelled (Dvl) protein prevents the degradation of β-catenin, which is involved in Wnt signaling pathway in the cytoplasm, and promotes the induction of gene expression by β-catenin translocated into the nucleus. The Wnt signaling pathway greatly participates in development, cell differentiation, and proliferation. Aberrations in the Wnt signaling pathway have been found in various tumors such as colorectal and breast cancer, and new therapeutic agents targeting the Wnt signaling pathway are being actively developed. Binding of Wnt, an extracellular signaling protein, to the Frizzled receptor (Fzd) on the cell membrane causes the binding of the Dvl protein to the cytoplasmic portion of Fzd, and inactivates the protein complex that degrades β-catenin. Then, β-catenin translocates into the nucleus, forms a complex with transcription factors, and induces expression of the target genes. The Dvl protein is a protein of about 700 residues consisting of multiple domains, and the PDZ domain of about 90 residues (Dvl_PDZ) is responsible for the function of binding to Fzd. Several small molecule inhibitors targeting Dvl_PDZ have been reported, but only one structural information has been available for the inhibitor complexed Dvl_PDZ. Therefore, the details of the binding mode between the various developed inhibitors and the ligand binding pocket of Dvl_PDZ is not sufficiently elucidated. In addition, there are three paralogs (Dvl1, 2, and 3) of human Dvls, and the only Dvl2 has been reported in the three-dimensional structure. We have been working on X-ray crystallographic analysis of Dvl_PDZ, and recently succeeded in determining the crystal structure of the human Dvl1 PDZ domain (hDvl1_PDZ) for the first time.

The structure reveals that hDvl1_PDZ forms a ring-shaped homo trimer by the interaction of the loop of adjacent molecules in the crystal (Fig. 1). This is the first example in the known oligomeric states of Dvl_PDZ. In Dvl_PDZ, it is well known that the ligand binding pocket exist between β2 and α2, and recognizes the C-terminal region or the side chains of acidic amino acids of the target protein. In the trimer structure of this study, the β2-β3 loop of the adjacent molecule interacts to the ligand binding pocket via the contact of the side chain of Asp272 and also a β-sheet like interactions between both the mainchains were also observed. It has been reported that the acidic amino acid side chains of the adjacent molecules interact with the ligand binding pocket and form a dimer structure. However, the molecular orientation was different from that of our trimer structure, suggesting that the ligand recognition mechanism of Dvl_PDZ may be diverse.

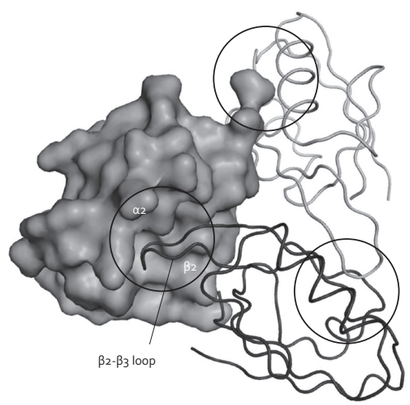

Fig.1 Crystal structure of the PDZ domain of human Dvl1. The ligand binding pocket where the β2-β3 loop of an adjacent molecule interacts is indicated by a black circle.

This work is performed in collaboration with Prof. Hiroaki of Nagoya University.

2. Mutational effects of Cys113 on structural dynamics of Pin1

Pin1 is a peptidyl-prolyl isomerase (PPIase) which catalyzes *cis*/*trans* isomerization of pS/pT-P bond. Its activity is related to various cellular functions including suppression of Alzheimer's disease. A cysteine residue C113 is known to be important for its PPIase activity; a mutation C113A reduced the activity by 130-fold. According to various nuclear magnetic resonance experiments for mutants of C113 and molecular dynamics (MD) simulation of wild-type Pin1, the protonation sate of Sγ of C113 regulates the hydrogen-bonding network of the dual-histidine motif (H59, H157) whose dynamics may affect substrate binding ability. However, it was still unclear why such local dynamic changes altered the PPIase activity of Pin1. In this study, we performed 500 ns of MD simulations of full-length wild-type Pin1 and C113A mutant in order to elucidate why the mutation C113A drastically reduced the PPIase activity of Pin1. The principal component analysis for both MD trajectories clearly elucidated that the mutation C113A suppressed the dynamics of Pin1 because it stabilized a hydrogen-bond between Nε of H59 and Oγ of S115 (Fig. 2). In the dynamics of wild-type protein, the phosphate binding loop (K63-S71) as well as the interdomain hinge showed the closed-open dynamics which correlated with the change of the hydrogen-bonding network of the dual-histidine motif. In contrast, in the dynamics of C113A mutant, the phosphate binding loop took only the closed conformation together with the interdomain hinge. Such closed-open dynamics must be essential for the PPIase activity of Pin1.

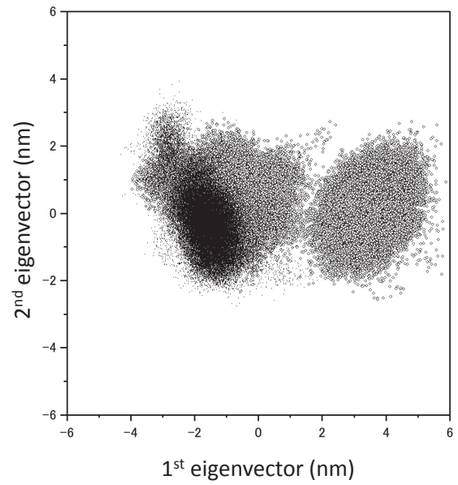

Fig.2 Principle component analysis of the whole snapshots consisting of wild-type and C113A trajectories. Projection of the wild-type (open circle) and C113A (black dot) trajectory onto the first 2 eigenvectors.

This work is performed in collaboration with Prof. Yonezawa (Kindai University).

3. Improvement in Protein Data Bank

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

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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. Pyruvate dehydrogenase PDH: an important regulator of cancer metabolism

Cancer cells often exhibit glycolytic metabolism in normoxia (also known as Warburg effect); however, the molecular mechanism of how such metabolism is formed has not been elucidated yet. We analyzed pyruvate dehydrogenase (PDH) in hypoxia and identified that PDH-E1β subunit is downregulated during chronic hypoxia. This downregulation is sustained upon reoxygenation, therefore causing glycolytic metabolism in breast cancer cells even under normoxic condition. Furthermore, knockdown of PDH-E1β in cancer cells caused a Warburg effect-like metabolism, which also points to the importance of PDH-E1β downregulation on inducing glycolytic metabolism in cancer cells. Finally, PDH-E1β-KD cells formed smaller tumors than the control cells in nude mice (**Figure 1**), indicating that sustained glycolytic metabolism is not sufficient to promote tumor growth, and implies a possibility of cancer cells also utilizing oxidative phosphorylation for their efficient energy production.

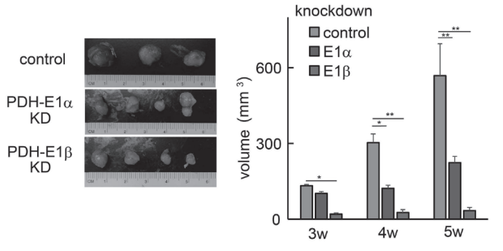

Figure 1　PDH knockdown suppresses tumor formation of breast cancer cell line
Knockdown of PDH suppressed the tumor forming ability of MB231 breast cancer cells in nude mice.

2. Gene regulation mediated by PDH

Recently, PDH was shown to localize in the nucleus. We also demonstrated that PDH localizes in the nucleus 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 downregulated under hypoxic condition. PDH promotes histone acetylation, and knockdown of PDH reduced the level of histone H3 acetylation. Similarly, prolonged hypoxic condition reduced histone H3 acetylation in breast cancer cell line and altered the expression of genes involved in cell death, immune response and hypoxic response. Gene expression and metabolism are central factors involved in cancer progression. PDH is a key molecule regulating both factors, and we further try to understand the molecular machinery of how it is regulated.

Publications

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2. Nakayama K.* and Kataoka N.* Regulation of Gene Expression under Hypoxic Conditions. ***Int. J. Mol. Sci.*** 20, E3278(1-15), **(2019)**.

Frontier Research Unit: Skeletal Molecular Pharmacology

Associate Professor　Yoichi Ezura

We aim to elucidate the molecular mechanisms of regulation for cellular responses in in vivo hard tissue related to the extracellular calcium regulation system to obtain useful knowledges for preventing and to cure intractable bone diseases.

1. Profilin1 in osteoclasts play important suppressive role in their movements and bone resorption

Profilin1, one of the regulators of actin filament polymerization assists elongation of straight actin filaments, and thus enhances locomotion of most mesenchymal cells including skeletal precursor cells, chondrocytes, osteoblasts and osteocytes. However, we found Profilin1 may have opposite function in osteoclasts, resulting in enhanced locomotion and bone resorption in its deficiency. Progressive deformities in developing long bones and facial bones were associated with altered distribution of functional osteoclasts. Such findings were consistent with the recent theory defining the structural dependency of the actin filament elongation by Profilin1. Our mutant mice demonstrating developmental osteolytic bone deformity would provide a useful model for congenital osteolysis of human disorders including the Paget's disease of bone.

2. Crnde is responsible for the PTH induced osteoblast proliferation possibly through Wnt/b-catenin signaling.

Parathyroid hormone (PTH) is known to promote osteoblast proliferation and bone formation through multiple pathways. By searching for the responsive factor to PTH induced osteoblast proliferation in culture, we focused on a long non-coding RNA named *Crnde*. By analyzing the mice deficient for *Crnde* via CRISPR-CAS9 system, we noticed a significant bone loss associated with a decreased number of osteoblasts and suppressed bone formation. Over-expression of *Crnde* resulted in an increase of osteoblast number and increase of osteogenic activity through Wnt / b-Catenin signal. We thus concluded that *Crnde* is one of the important regulators of osteoblast proliferation by PTH, and we are now assuming relationships to certain types of genetic diseases.

3. Heterotopic ossification of ligament/tendon cells is mediated through oxidative stress induced extracellular ATP and adenosine.

The ossification of the posterior longitudinal ligament (OPLL) is one of the intractable diseases defined in Japan. To understand the molecular bases for the pathogenesis of OPLL, we focused on a gene encoding an adenosine transporter localized on cell membrane *SLC29A1*. Since the *Slc29a1*-deficient mice were shown to demonstrate ectopic spinal ligament bone formation by aging, we assumed extracellular ATP in response to aging- and trauma-based oxidative stress is important. Elucidation of the mechanism of extracellular ATP-derived adenosine-mediated tendon cell ossification using inhibitors for the transporter etc., in TT-D6 cells and primary cultured cells derived from the mouse flexor tendon of digits.

Publications

1. Shirakawa J, Kajikawa S, Bottcher RT, Costell M, Izu Y, Hayata T, Noda M, Ezura Y. Profilin 1 negatively regulates osteoclast migration in postnatal skeletal growth, remodeling and homeostasis in mice JBMR-Plus e10130 (2019 Jan).
4. Mulati M, Kobayashi Y, Takahashi A, Numata H, Saito M, Hiraoka Y, Ochi H, Sato S, Ezura Y, Yuasa M, Hirai T, Yoshii T, Okawa A, Inose H. The long noncoding RNA Crnde regulates osteoblast proliferation through the Wnt/b-catenin signaling pathway in mice. Bone 130: 115076 (2020 Jan)

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 biomarker which reflects neuronal necrosis at the ultra-early stage of Alzheimer's disease
- Discovery of YAP protein as a new therapeutic target for Alzheimer's disease

【Biochemical Pathophysiology】

- Identification of cancer-promoting "oncolipids" and proinflammatory lipids

【Pathological Cell Biology】

- Elucidation of the mechanism of Ulk1-mediated alternative autophagy
- Discovery of alternative anoikis

【Developmental and Regenerative Biology】

- Elucidation of the mechanism of cellular clock synchronization in zebrafish

【Stem Cell Biology】

- Elucidation of the mechanisms of skin aging

【Immunology】

- Elucidation of the role of inhibitory B cell co-receptor CD72 in the regulation of autoantibody production in systemic lupus erythematosus (SLE)
- Discovery of genetic association of Guillain-Barre syndrome with Siglec-10

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

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 ability to diagnose AD at an early stage is eagerly anticipated, especially after clinical trials of anti-Aβ antibodies and γ-/β-secretase inhibitors in post-onset patients proved disappointing. A deeper understanding of MCI could play a pivotal role in the development of new therapeutic strategies for AD. Despite the importance of MCI, the pathological and molecular evaluation remains insufficient especially from the aspect of chronological change of neuronal function and cell death. Accordingly, no efficient single biomarker directly reflecting disease activity in MCI has yet been reported.

Cutting-edge techniques, including comprehensive analyses, have identified molecules in addition to Aβ and tau that could be targeted for therapeutic intervention at the early stage of AD. For instance, comparison of neuroimaging and transcriptome data revealed that a genetic profile of lipid metabolism centered by APOE affects propagation patterns of both Aβ and tau in the brain. In another study, a meta-analysis of functional genomic data from AD showed that YAP, a co-transcriptional factor that regulates cell death and survival by binding to the different transcription factors p73 and TEA domain family member 1 (TEAD), is positioned at the center of the molecular network of AD. Elevated activity of TEAD mediated by YAP has been implicated in cell proliferation, differentiation, and survival, whereas elevated p73 activity and reduced TEAD activity promote apoptosis and necrosis, respectively.

Previously, we performed a comprehensive phosphoproteome analysis of four strains of AD model mice and human postmortem AD brains, and discovered three proteins whose phosphorylation state is altered at a very early stage before extracellular amyloid aggregates. One such protein is MARCKS, which anchors the actin cytoskeleton to the plasma membrane and plays a critical role in stabilizing the post-synaptic structure of dendritic spines. Phosphorylation of MARCKS at Ser46 decreases its affinity for actin and destabilizes dendritic spines. High mobility group box-1 (HMGB1) contributes to the MARCKS phosphorylation via Toll-like receptor 4 (TLR4) since blockade of HMGB1–TLR4 binding with monoclonal anti-HMGB1 antibodies suppresses the phosphorylation of MARCKS at Ser46, stabilizes dendritic spines, and rescues cognitive impairment in AD model mice. Given that HMGB1 is released from necrotic cells, it remains unclear how MARCKS phosphorylation, which occurs at the early stage of AD pathology, is connected to neuronal cell death, which is believed to occur at a relatively late stage.

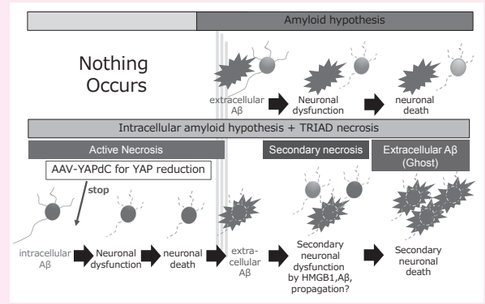
In the current study, we found that HMGB1 levels were remarkably elevated in CSF of mild cognitive impairment (MCI), but not so elevated in AD patients. Consistent with this, active neuronal necrosis revealed by our original marker, myristoylated alanine-rich C-kinase substrate phosphorylated at serine 46 (pSer46-MARCKS), increased to the greatest extent during preclinical stages of AD mouse models and human MCI patients. Postmortem brains of MCI rather than symptomatic AD patients reveal a remarkable increase of necrosis. In vivo imaging reveals instability of endoplasmic reticulum (ER) in mouse AD models and genome-edited human AD iPS cell-derived neurons, which were reminiscent of transcriptional repression-induced atypical cell death (TRIAD). In addition, we showed that the observed necrosis was caused by a deficiency of YAP, resulting in suppression of the transcriptional activity of TEAD, the final effector molecule of the Hippo pathway. The level of nuclear Yes-associated protein (YAP) is remarkably decreased in such neurons under AD pathology due to the sequestration into cytoplasmic amyloid beta (Aβ) aggregates, supporting the feature of YAP-dependent necrosis. Suppression of early-stage neuronal death by AAV-YAPdeltaC reduces the later-stage extracellular Aβ burden and cognitive impairment. These findings unravel the occurrence of cell death at the early stage in AD, suggesting that preclinical/prodromal YAP-dependent neuronal necrosis represents a target for AD therapeutics.

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

Tanaka, H., Homma, H., Fujita, K., Kondo, K., Yamada, S., Jin, X., Waragai, M., Ohtomo, G., Iwata, A., Tagawa, K., Atsuta, N., Katsuno, M., Tomita, N., Furukawa, K., Saito, Y., Saito, T., Ichise, A., Shibata, S., Arai, H., Saido, T., Sudol, M., Muramatsu, S., Okano, H., Mufson, E. J., Sobue, G., Murayama, S. & Okazawa, H. (2020) YAP-dependent necrosis occurs in early stages of Alzheimer's disease and regulates mouse model pathology. *Nat. Commun.* 24 January 2020, 11 (1), 507. doi: 10.1038/s41467-020-14353-6

Rahman, S. K., Okazawa, H. & Chen, Y. W. (2019) Frameshift PQBP-1 mutants K192Sfs*7 and R153Sfs*41 implicated in X-linked intellectual disability form stable dimers. *J. Struct. Biol.* 1 June 2019, 206 (3), 305–313. doi: 10.1016/J.JSB.2019.04.003

Bannai, T., Mano, T., Chen, X., Ohtomo, G., Ohtomo, R., Tsuchida, T., Koshi-Mano, K., Hashimoto, T., Okazawa, H., Iwatsubo, T., Tsuji, S., Toda, T. & Iwata, A. (2019) Chronic cerebral hypoperfusion shifts the equilibrium of amyloid β oligomers to aggregation-prone species with higher molecular weight. *Sci. Rep.* 26 February 2019, 9 (1), 2827. doi: 10.1038/s41598-019-39494-7

Inoue, S., Hayashi, K., Fujita, K., Tagawa, K., Okazawa, H., Kubo, K.-I. & Nakajima, K. (2019) Drebrin-like (Dbnl) Controls Neuronal Migration via Regulating N-Cadherin Expression in the Developing Cerebral Cortex. *J. Neurosci.* 23 January 2019, 39 (4), 678–691. doi: 10.1523/JNEUROSCI.1634-18.2018

Chen, X., Kondo, K. & Okazawa, H. (2019). Methods to Image Macroautophagy in the Brain In Vivo. *Methods in Molecular Biology,* 2019, 1880, 529–534. doi: 10.1007/978-1-4939-8873-0_33

Department of Biochemical Pathophysiology

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

Molecules that shape organisms and support their activities can be roughly classified into three layers. From the top of the flow of information, they are genes, proteins, and metabolites. Metabolites are in a position that directly controls phenotype, the states of an organism. They fluctuate quantitatively and qualitatively in our bodies due to the effects of genes, proteins, diets, drugs, and symbiotic bacteria. Lipids are the metabolites that are poorly soluble in water, and are used for compartmentalization of cells by membrane formation, energy storage, and signal transduction inside and outside cells. Research on lipids is yet to be advanced, as analytical methods have not been well-generalized compared to those for genes and proteins. That being the case, we believe that this is a research subject that will present new principles of life and provide a lot of knowledge to help overcome diseases. In fact, with the improvement of mass spectrometry technologies, the identification of new bioactive lipid molecules has recently been in succession. Our laboratory has also identified lipids with novel structure that fluctuate in cancers, neurological diseases, and inflammatory diseases. We are working on the identification of their synthesizing and degrading enzymes, physiological functions, and the molecular mechanisms of their actions.

〈**Research Projects**〉

1. Developing predictive markers for cancer therapy by lipidomics

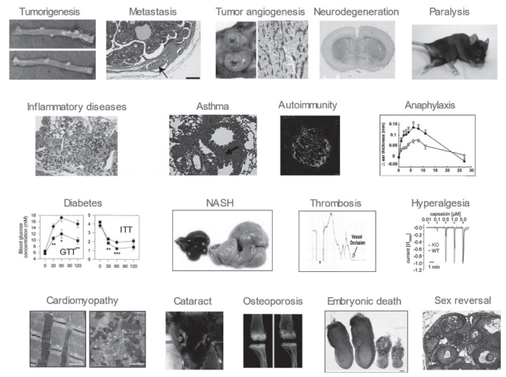
Phosphoinositide 3-kinases (PI3Ks) phosphorylate phosphatidylinositol 4,5-bisphosphate (PIP2) to produce phosphatidylinositol 3,4,5-trisphosphate (PIP3). Conversely, PTEN is the phosphatase that dephosphorylates the same position of the hydroxyl where PI3K phosphorylates, and converts PIP3 back to PIP2. 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 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 phosphoinositides 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 PIP3 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. Polarized PtdIns(4,5)P2 distribution mediated by a voltage-sensing phosphatase (VSP) regulates sperm motility. Kawai T, Miyata H, Nakanishi H, Sakata S, Morioka S, Sasaki J, Watanabe M, Sakimura K, Fujimoto T, Sasaki T, Ikawa M, Okamura Y. Proc Natl Acad Sci U S A. 116(51):26020-26028, 2019
2. Increased fatty acyl saturation of phosphatidylinositol phosphates in prostate cancer progression. Koizumi A, Narita S, Nakanishi H, Ishikawa M, Eguchi S, Kimura H, Takasuga S, Huang M, Inoue T, Sasaki J, Yoshioka T, Habuchi T, Sasaki T. Sci Rep. 9(1):13257, 2019
3. A Negative Feedback Loop Regulates Integrin Inactivation and Promotes Neutrophil Recruitment to Inflammatory Sites. McCormick B, Craig HE, Chu JY, Carlin LM, Canel M, Wollweber F, Toivakka M, Michael M, Astier AL, Norton L, Lilja J, Felton JM, Sasaki T, Ivaska J, Hers I, Dransfield I, Rossi AG, Vermeren S. J Immunol. 203(6):1579-1588, 2019
4. A Peptide Derived from Phosphoinositide 3-kinase Inhibits Endocytosis and Influenza Virus Infection. Fujioka Y, Satoh AO, Horiuchi K, Fujioka M, Tsutsumi K, Sasaki J, Nepal P, Kashiwagi S, Paudel S, Nishide S, Nanbo A, Sasaki T, Ohba Y. Cell Struct Funct. 44(1):61-74, 2019
5. Lysophosphatidylinositol-acyltransferase-1 is involved in cytosolic Ca2+ oscillations in macrophages. Takemasu S, Ito M, Morioka S, Nigorikawa K, Kofuji S, Takasuga S, Eguchi S, Nakanishi H, Matsuoka I, Sasaki J, Sasaki T, Hazeki K. Genes Cells. 24(5):366-376, 2019

Department of Pathological Cell Biology

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	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 a novel phosphorylation site of Ulk1 at Ser^{746}, which is phosphorylated during genotoxic stress-induced alternative autophagy. Phospho-$Ulk1^{746}$localizes exclusively on the Golgi and is required for alternative autophagy, but not canonical autophagy. We also identified RIPK3 as the kinase responsible for genotoxic stress-induced $Ulk1^{746}$ phosphorylation, because RIPK3 interacts with and phosphorylates Ulk1 at Ser^{746}, and loss of RIPK3 abolished $Ulk1^{746}$ phosphorylation. We also showed that $Ulk1^{637}$ dephosphorylation mediated by P53 and protein phosphatase, Mg^{2+}/Mn^{2+} dependent 1D is required for RIPK3-dependent $Ulk1^{746}$ phosphorylation and alternative autophagy. These findings indicate that RIPK3-dependent $Ulk1^{746}$ phosphorylation on the Golgi plays a pivotal role in genotoxic stress-induced alternative 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 discovered novel form cell death, namely alternative anoikis. Anoikis is a cell death induced by the detachment from the extracellular matrix, and has long been considered to be mediated by apoptosis. However, recently, we found that anoikis is not prevented by the addition of caspase inhibitors. Apoptosis-resistant Bax/Bak-deficient cells also died by anoikis, indicating the existence of non-apoptotic anoikis. We named this type of anoikis as 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.

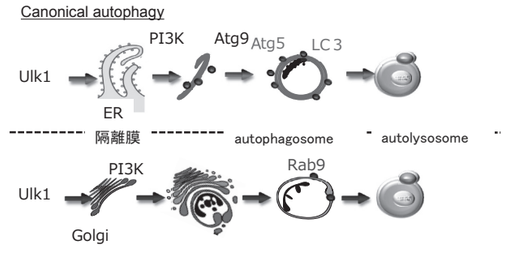

Figure1. Hypothetical model of autophagy.
There are at least two modes of autophagy, i.e. canonical and alternative autophagy. Canonical autophagy requires Atg5 and is originated from the ER membrane. In contrast, alternative autophagy occurs independently of Atg5 and is originated from the Golgi membrane.

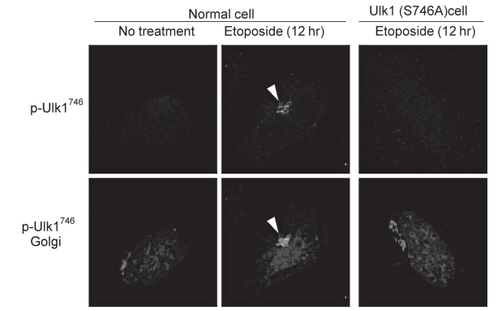

Figure2. Induction of the Golgi localization of p-$Ulk1^{746}$ by etoposide treatment.
The indicated MEFs were treated with etoposide, and immunostained with anti-p-$Ulk1^{746}$ and anti-GS28 antibodies. Representative images of p-$Ulk1^{746}$ (upper panels) and merged images are shown.

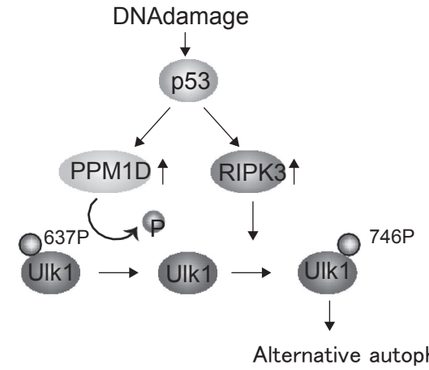

Figure3. Schematic model of the RIPK3-dependent alternative autophagy.
Genotoxic stress induces Ulk1 dephosphorylation at Ser^{637} in a p53/PPM1D-dependent manner. The dephosphorylated Ulk1 is then phosphorylated at Ser^{746} by RIPK3 and induces alternative autophagy.

List of Publications

[Original paper]

1. Beclin 1 regulates recycling endosome and is required for skin development in mice. *S. Noguchi, S. Honda, T. Saitoh, H. Matsumura, E. Nishimura, S. Akira. S. Shimizu.* **Communications Biology** 2: article No. 37, 2019

2. Prediction of intracellular targets of a small compound by analyzing peptides presented on MHC class I. *Y. Sugimoto, M. Murohashi, S. Arakawa, S. Honda, S. Shimizu.* **BBRC** 508, 480-486, 2019.

3. 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

4. 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** in press.

5. Identification of a novel phosphorylation site of Ulk1 that is required for genotoxic stress-induced alternative autophagy. *S. Torii, H. Yamaguchi, A. Nakanishi, S. Arakawa, S. Honda, K. Moriwaki, H. Nakano, S. Shimizu.* **Nature Communications** *in press*

Department of Developmental and Regenerative Biology

Professor	Hiroshi Nishina, Ph.D.
Lecturer	Kengo Honma, Ph. D., Satoshi Kofuji, Ph. D.
Assistant Professor	Erika Ishihara, Ph.D.
Research Assistant Professor	Yukari Mori, M.D., Ph.D.

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

1. SAPK/JNK Signaling Pathway

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

2. Hippo Signaling Pathway

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

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

3. Circadian Clock

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

Highlight

The circadian clock generates behavioral rhythms to maximize an organism's physiological efficiency. Light induces the formation of these rhythms by synchronizing cellular clocks. Here, we investigated the roles of zPER2, zCRY1a and zCRY2a in regulating locomotor activity and behavioral rhythms. Overall, our results suggest that zPER2, zCRY1a and zCRY2a help to synchronize cellular clocks in a light-dependent manner, thus contributing to behavioral rhythm formation in zebrafish. Further, zPER2 and zCRY1a regulate total physical activity, likely via regulating cellular energy metabolism. Therefore, these circadian clock components regulate the rhythmicity and amount of locomotor behavior.

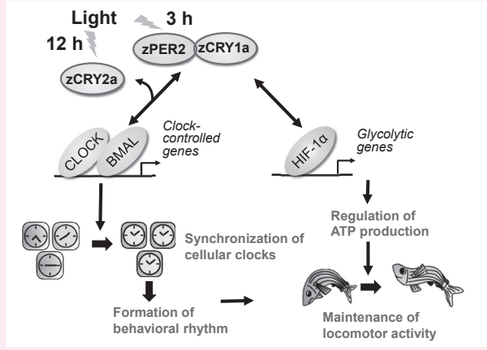

Fig.1.A proposal model depicting the mechanism underlying zPER2, zCRY1a and zCRY2a-mediated regulation of behavior rhythm and locomotor activity.

Publications

1. Jun Hirayama, Yikelamu Alifu, Rin Hamabe, Sho Yamaguchi, Jun Tomita, Yusuke Maruyama, Yoichi Asaoka, Ken-ichi Nakahama, Teruya Tamaru, Ken Takamatsu, Nobuhiko Takamatsu, Atsuhiko Hattori, Sachiko Nishina, Noriyuki Azuma, Atsuo Kawahara, Kazuhiko Kume*, Hiroshi Nishina (2019) The clock components *Period2*, *Cryptochrome1a*, and *Cryptochrome2a* are required for forming light-dependent behavioral rhythms and/or maintaining total activity levels in zebrafish. ***Scientific Reports*** 9, 196.

2. Miki Nishio, Yousuke Miyachi, Junji Otani, Shoji Tane, Hirofumi Omori, Fumihito Ueda, Hideru Togashi, Takehiko Sasaki, Tak Wah Mak, Kazuwa Nakao, Yasuyuki Fujita, Hiroshi Nishina, Tomohiko Maehama and Akira Suzuki (2019) Hippo pathway controls cell adhesion and context-dependent cell competition to influence skin engraftment efficiency. ***FASEB J*** doi: 10.1096/fj.201802005R

3. Ryo Goto, Kenya Kamimura, Yoko Shinagawa-Kobayashi, Norihiro Sakai, Takuro Nagoya, Yusuke Niwa, Masayoshi Ko, Kohei Ogawa, Ryosuke Inoue, Takeshi Yokoo, Akira Sakamaki, Hiroteru Kamimura, Satoshi Abe, Hiroshi Nishina and Shuji Terai (2019) Inhibition of sodium glucose cotransporter 2 (SGLT2) delays liver fibrosis in a medaka model of nonalcoholic steatohepatitis (NASH). ***FEBS Open Bio*** doi:10.1002/2211-5463.12598

4. Takuro Nagoya, Kenya Kamimura, Ryo Goto, Yoko Shinagawa-Kobayashi, Yusuke Niwa, Atsushi Kimura, Norihiro Sakai, Masayoshi Ko, Hiroshi Nishina, Shuji Terai (2019) Inhibition of sodium-glucose cotransporter 2 ameliorated renal injury in a novel medaka model of nonalcoholic steatohepatitis-related kidney disease. ***FEBS Open Bio*** doi:10.1002/2211-5463.12734

Department of Stem Cell Biology

Professor	Emi K. Nishimura, M.D., Ph. D.
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Assistant Professor	Hiroyuki Matsumura, Ph. D.
Project Assistant Professor	Yasuaki Mohri, Ph. D., Hironobu Morinaga, Ph. D., Kyosuke Asakawa, Ph. D.,Liu Nan, M.D., 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 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 non-hair-bearing skin areas (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).

2) Mechanisms of stem cell maintenance

The underlying mechanisms of stem cell maintenance are a fundamental issue in stem cell biology and medicine. We previously found 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). The niche functions of HFSCs are mediated by extrinsic niche factors, including transforming growth factor β (TGF-β), that are secreted from HFSCs to maintain McSCs in a quiescent and immature state. Meanwhile, intrinsic defects in stem cells, such as those caused by *Mitf* or *Bcl2* deficiencies in mice, also induce the depletion of McSCs, which leads to the progressive expression of the hair graying phenotype. Therefore, we concluded that the incomplete maintenance of McSCs either by defective signaling from the stem cell niche or by intrinsic defects in stem cells, results in an insufficient supply of mature melanocytes for hair pigmentation in mice expressing the progressive hair graying phenotype (Figure 1).

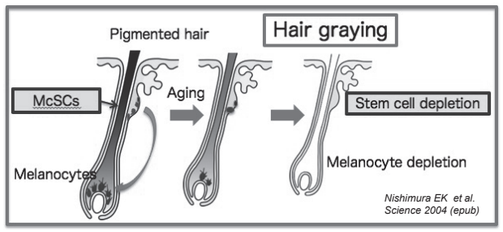

Figure 1: Aging and genomic stress abrogates the self-renewal of McSCs causing hair graying.

3) 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, 2016). We are currently trying to identify the stem cell division program for organ aging.

4) 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. We have performed *in vivo* clonal analysis in mice by focusing on the expression of the hemidesmosomal protein COL17A1 by epidermal stem cells. Those studies 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.

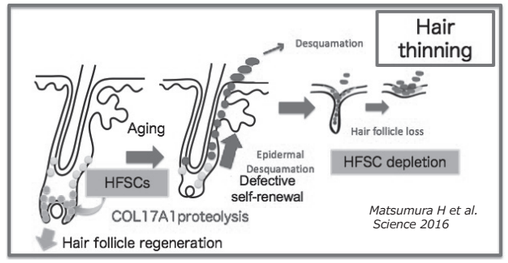

Figure 2: Dynamic elimination of aged stem cells causes hair follicle aging.

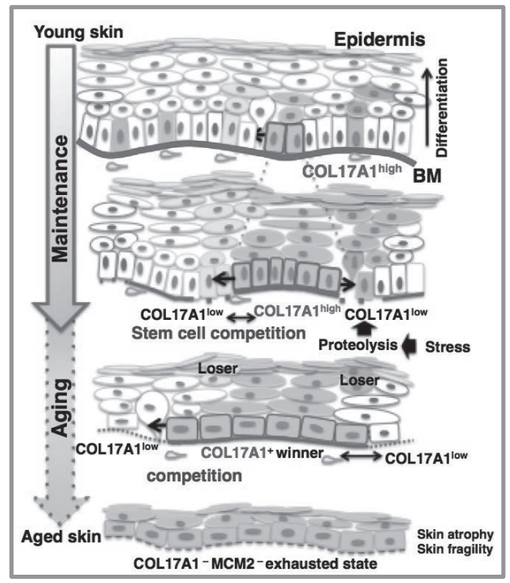

Figure 3: Stem cell competition orchestrates skin homeostasis and aging. SCD: symmetric cell division; ACD : asymmetric cell division.

Annual publications

Liu N, Matsumura H, Kato T, Ichinose S, Takada A, Namiki T, Asakawa K, Morinaga H, Mohri Y, De Arcangelis, Georges-Labouesse E, Nanba D, Nishimura EK
Stem cell competition orchestrates skin homeostasis and ageing.
Nature, 568(7752):344-350, **2019** doi: 10.1038/s41586-019-1085-7.

Muraguchi T, Nanb D, Nishimura EK., Tashiro T
IGF-1R deficiency in human keratinocytes disrupts epidermal homeostasiss and stem cell maintenance
J Dermatol Sci., 94(2):298-305, **2019** doi: 10.1016/j.jdermsci.2019.05.001.

Kinoshita K, Munesue T, Toki F, Isshiki M, Higashiyama S, Barrandon Y, Nishimura EK, Yanagihara Y, Nanba D
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Sci Rep. 9(1):18725, 2019 doi: 10.1038/s41598-019-55279-4.

Noguchi S, Honda S, Saitoh T, Matsumura H, Nishimura E, Akira S, Shimizu S.
Beclin 1 regulates recycling endosome and is required for skin development in mice.
Commun Biol. 2:37. **2019** doi: 10.1038/s42003-018-0279-0.

Department of Immunology

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Researcher	Nazim Medhzidov, Ph.D., Feng Yang-yang, Ph.D.

Immune responses to non-protein antigens play crucial roles in host defense against pathogens, and autoimmune diseases. The mechanisms for immune responses to non-protien antigens are distinct from those to protein antigens, but are largely unknown. The aims of our research are to elucidate the mechanisms for antibody responses to non-protein antigens, and to develop novel drugs for autoimmune diseases and cancer immunotherapy by regulating antibody responses. Followings are our research subjects.

1) Elucidation of the mechanisms for humoral immune responses to polysaccharide antigens.
2) Elucidation of the mechanisms for autoantibody production in lupus and immuno-neurological disorders.
3) Elucidation of the role of glycan signals in the regulation of B lymphocyte (B cell) activation
4) Elucidation of the Role of endosomal signaling in B cell activation
5) Development of novel drugs for autoimmune diseases by regulating regulatory B cells.
6) Development of therapeutic vaccines that substitute for therapeutic antibodies

1. Inhibition of development of systemic lupus erythematosus by the inhibitory B cell co-receptor CD72.

Systemic lupus erythematosus (SLE) is a prototype of systemic autoimmune diseases, and characterized by production of autoantibodies to various nuclear components containing nucleic acids (NAs). Immune complexes formed by self-antigens and autoantibodies precipitate on the vasculature. Inflammation caused by the immune complexes play a central role in the pathogenesis of SLE.

Immune cells including B lymphocytes (B cells) express various receptors that recognize NAs derived from microbes especially virus. These NA sensors play a crucial role in host defense against microbes. Among NA sensors, TLR7, TLR8 and TLR9 are located in endosome, and TLR7 and TLR9 are known to recognize self-NAs as well as microbial NAs. TLR7 recognizes the lupus self-antigen Sm/RNP, and plays an essential role in development of SLE. When NA-containing self-antigens such as Sm/RNP are recognized by self-reactive B cells, self-antigens are internalized and transported to endosome, where NAs activate B cells via TLRs and induce autoantibody production.

We have demonstrated that the B cell inhibitory co-receptor CD72 suppresses development of SLE (reviewed in Tsubata 2019). We further demonstrated that CD72 inhibits B cell responses to Sm/RNP and production of anti-Sm/RNP autoantibody by specifically recognizing Sm/RNP as a ligand. However, patients with SLE produce autoantibodies to various NA-containing self-antigens other than Sm/RNP. We therefore addressed whether CD72 recognizes other self-antigens using autoantigen microarray in collaboration with Dr. Li at University Texas Southwestern. We then demonstrated that CD72 recognizes various other NA-containing self-antigens although CD72 does not recognize NAs themselves. Thus, CD72 appears to suppress autoimmune responses to NA-containing self-antigens without suppressing immune response to microbes by recognizing NA-related self-antigens but not NAs, thereby inhibiting development of SLE (Figure 1).

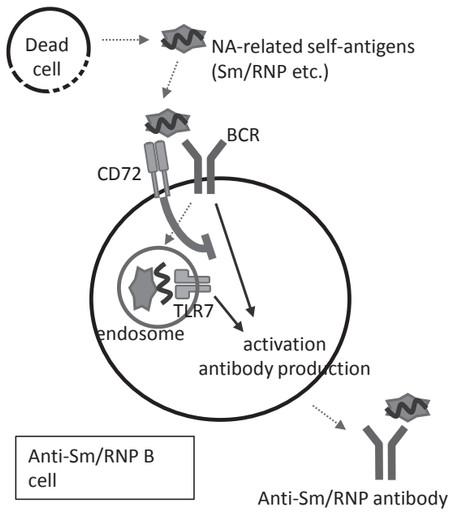

Figure1. Regulation of B cell responses to nucleic acid (NA)-related self-antigens by the inhibitory B cell co-receptors CD72.
When NA-containing self-antigens such as Sm/RNP derived from dead cells are recognized by self-reactive B cells as antigens, signaling through B cell antigen receptor (BCR) is generated. NA-containing self-antigens bound by BCR are internalized and transported to endosome, where self-antigen-derived NAs stimulate TLRs. In the presence of BCR signaling and TLR signaling, self-reactive B cells are activated and produce autoantibodies. CD72 is recruited to BCR by recognizing NA-containing self-antigens bound to BCR, and inhibits BCR signaling. As a consequence, activation of self-reactive B cells and autoantibody production are inhibited. Originally published in Proceedings of the Japan Academy, Series B, Physical and Biological Sciences, Tsubata (2018).

Highlight

Suppression of reactive oxygen species (ROS) augments differentiation of B cells to plasma cells.

We have demonstrated that ligation of B cell antigen receptor (BCR) induces production of reactive oxygen species (ROS) by activating NADPH oxidases. To address the role of ROS in the regulation of differentiation of B cells to antibody-producing cells (plasma cells), we stimulated mouse primary B cells with cytokines and anti-IgM antibody that ligates BCR in the presence or absence of the ROS scavenger N-acetylcysteine (NAC). NAC inhibited proliferation of B cells almost completely, but markedly augments differentiation of B cells to plasma cells and antibody production (Figure 2). This result clearly indicates that ROS is required for proliferation of B cells stimulated with antigens but suppresses differentiation to plasma cells. In various cell types including muscle cells and neurons, it was demonstrated that cell cycling inhibits cell differentiation. Our results suggest that cell cycling inhibits differentiation of B cells. We are now trying to identify the molecular mechanisms for the regulation of cell differentiation by cell cycling in B cells. Our results also suggest that ROS scavengers can augment antibody production. This finding may be applicable to development of novel vaccine with higher efficacy.

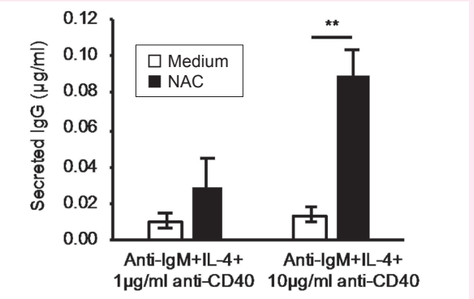

Figure2. ROS scavenging augments antibody production from B cells.
Mouse primary B cells are stimulated with anti-IgM antibody, IL-4 and anti-CD40 antibody in the presence or absence of the ROS scavenger N-acetyl cysteine (NAC), and the concentration of IgG in the culture supernatant was measured. NAC strongly augments IgG production although B cell proliferation is almost completely inhibited. Originally published in *The Journal of Immunology*. Feng Y.-y. et al.. 2019. J. Immunol. 202: 2546-2557. Copyright © [2019] The American Association of Immunologists, Inc.

Publications

[original papers]

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[Reviews and Book Chapters]

1. Tsubata, T. (2019): CD72 is a negative regulator of B cell responses to nuclear lupus self-antigens and development of SLE. *Immune Netw.* 19:e1.
2. Tsubata, T (2019): Development of Siglec Regulators. In “Glycoscience: Basic Science to Applications” ed. by Naoyuki Taniguchi, Tamao Endo, Jun Hirabayashi, Shoko Nishihara, Kenji Kadomatsu, Kazunari Akiyoshi and Kiyoko F. Aoki-Kinoshita, p. 170-171, Springer.
3. Alborzian Deh Sheikh, A., Akatsu, C. and Tsubata, T. (2020): Identification of Siglec Cis-ligands by Proximity Labeling. In “Lectin Purification and Analysis: Methods and Protocols” ed. by Jun Hirabayashi, Springer (in press).
4. Tsubata, T. (2019): Inhibitory B cell co-receptors and autoimmune diseases. *Immunol. Med.* 2(3): 108-116.

Division of Medical Genomics

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

【Molecular Cytogenetics】

The principal aim of the Department of Molecular Cytogenetics is to understand the molecular mechanisms and establish practically useful modalities for the diagnosis and therapy of personalized medicine for cancer and genetic diseases including intellectual disability and/or intellectual disability (ID/MCA), with the purpose of addressing unmet medical needs.

1. We Identified novel tumor suppressive microRNAs (TS-miRs) by using cell-based screening with a total of 2,565 human *miR* mimics library. Among them some TS-*miRs* that directly suppressed *BRD4* by binding to its 3'UTR and showed *in vivo* therapeutic effects of candidate *miRs* using a xenograft mouse model. These TS-*miRs* might be promising candidates for the development of miRNA-based cancer therapy.
2. Drug delivery system (DDS) is the critical for the implementation of nucleic acid-based therapy. Recently, we validated the therapeutic potential of LNP (lipid nanoparticles)-mediated delivery of *miR-634* for cancer therapy. By using systemic administration of *miR-634*-LNP with tail vein-injection, we confirmed not only efficient delivery of *miR-634* into tumor cells and inhibition of expression of target genes, but also a remarkable antitumor effect in the group treated with *miR-634*-LNP. The LNP-mediated delivery of *miR-634* might potentially be useful for cancer therapy.
3. Since 2005, we have investigated 645 subjects with undiagnosed ID/MCA recruited from 23 institutes in Japan. Among them, we identified pathogenic CNVs in 155 cases (24%), and then in residual pathogenic CNV-negative cases we identified pathogenic nucleotide variants in 20 cases (19%) using a 75-gene custom panel. Recently, we identified novel genes responsible for ID/MCA in two cases through whole-exome sequencing in trios of six cases.

【Molecular Genetics】

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

1. We sought novel synthetic lethal interactions between BRCA1/2 and chemical compounds provided by Chemical Biology Screening Center at TMDU.
2. We focused on breast cancer invasion, and analyzed the mechanism for disruption of the breast duct and basement membrane by estrogen.
3. Hereditary breast and ovarian cancer (HBOC) has dysfunctional DNA homologous recombination repair, and shows sensitive response to PARP inhibitors. However, various types of acquired chemoresistance mechanisms were reported. We screened chemical compounds to explore novel factors to sensitize tumor cells combined with PARP inhibitor.

【Molecular Epidemiology】

1. We established birth cohort, BC-GENIST, to study health-related epigenome markers in collaboration with TMDU hospital.
2. We newly identified the cryptic heterogeneity in fetal growth velocity by latent class trajectory analysis.
3. We have found that the effect of gestational weight gain on fetal growth was smaller in lower quantiles of the birth weight distribution, prompting a reconsideration of the risks and appropriateness of a uniform recommendation for weight gain increase.
4. We reproduced to show the association between HIF3A methylation and birth weight, using 300 actual blood spot cards archived after the newborn screening test.

【Epigenetics】

1. We reported the existence of sushi-ichi-related retrotransposon homologue family of genes (SIRH family genes) and demonstrated that they play essential roles in placenta, such as *Peg10*, *Peg11/Rtl1* and *Sirh7*, or in brain, such as *Sirh11*, *Sirh3* and *Sirh8* in a eutherian-specific manner.
2. We have developed a new method for induce heart-like structure (heart organoid) from mouse ES cells and human iPS cells. In this method, heart organoid develops mimicking normal heart development in vivo.
3. We have developed a new method for screening novel genes derived from human endogenous retroviruses (HERVs).

【Medical Science Mathematics】

1. We have developed a new scheme to analyze non-image data, e.g. gene-expression profiles, with deep neural network by converting the data into an image-like form. Applying this method to several tasks, e.g. cancer classification, we found that it can perform much better than traditional machine learning methods. This technique is generally applicable for extracting features to classify samples in biomedicine.
2. We have developed original methodologies: network topology analysis for identifying key molecules for disease and drug-response and constructing their prediction models, and a highly accurate model for predicting drug-toxicity by using machine learning to combine features from omic and physicochemical properties. With these, we got the top-winner awards in the DREAM challenge.
3. In addition, we achieved: dementia subtype classification and risk prediction, mendelian disease analysis with whole exome sequencing, single cell clustering method for small sample set, and a GWAS textbook publication.

【Genomic Function and Diversity】

1. To dissect pathological mechanism of complex traits, we integrated the disease GWAS data with expression QTL and splicing QTL data, and we comprehensively identified disease-associated alternative isoforms.
2. By re-sequencing the GWAS genes in rheumatoid arthritis patients, we identified accumulation of rare coding variants in TYK2 gene that influenced inflammatory cytokine signals.
3. To further seek disease susceptible loci for systemic lupus erythematosus, we are performing GWAS meta-analysis in world-wide collaboration.

Department of Molecular Cytogenetics

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Associate Professor	Jun Inoue, Ph.D.
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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 establish practically useful modalities for the diagnosis and therapy of personalized medicine for cancer and genetic diseases including intellectual disability and/or intellectual disability (ID/MCA), with the purpose of addressing unmet medical needs.

I. Development of microRNA-based cancer therapeutics

1) Development of anticancer drug with tumor-suppressive *miR-634* as a drug seed

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. Recently, we validated the therapeutic potential of LNP (lipid nanoparticles)-mediated delivery of *miR-634* for cancer therapy. A *miR-634*-LNP was created by incorporating synthetic double-stranded *miR-634* into LNP (provided by Eisai Co., Ltd.), and then BxPC-3 tumor-bearing mice were systemically treated with miR-634-LNP in an experimental manner. The results showed not only efficient delivery of *miR-634* into tumor cells and inhibition of expression of target genes but also a remarkable antitumor effect in the group treated with *miR-634*-LNP compared to the control group. These findings suggest that LNP-mediated delivery of *miR-634* can potentially be used for cancer therapy (Gokita K et al. Mol Therapy - Nucleic Acids. 2019).

2) Exploration of novel TS-*miRs*

To investigate novel 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 cancer cells. We identified several TS-*miRs* that directly suppressed *BRD4* by binding to its 3'UTR. To reveal the tumor-suppressive mechanisms of these candidate *miRs*, we further explored the target genes other than *BRD4* by gene expression analysis. We also examined the *in vivo* therapeutic effects of candidate *miRs* using a xenograft mouse model. These TS-*miRs* might be candidates for the development of miRNA-based cancer therapeutics.

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

1) Development of angiogenesis-targeting therapy

Angiogenesis is important for cancer metastasis because new vessels in tumor or around tumor provide the principal route by which tumor cells exit the primary tumor site and enter the circulation. However, the detailed mechanisms underlying these processes remain poorly understood, and useful molecular target drugs against metastasis remain inadequate so far. Recently, we identified some compounds which have the potential of inhibiting *VEGFA* (vascular endothelial growth factor A) expression by cell-based screening assay using chemical compound libraries. Interestingly, almost all of these compounds can inhibit the same signaling pathway. These results suggested that these candidate compounds might have the potential of anti-angiogenic therapy for cancer.

2) Development of cancer metabolism-targeting therapy

By using an siRNA library for 192 metabolism-related genes, which are involved with glycolysis, OXPHOS, lipid metabolism, amino acid metabolism, and autophagy, we found novel therapeutic targets related with cancer metabolism. This finding may lead to the development of precision cancer medicine based on the metabolism of cancer cells.

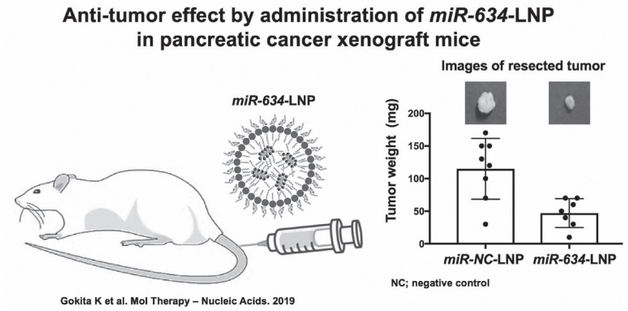
III. Molecular investigation of congenital disorders

1) Genetic causes of intellectual disability and multiple congenital anomalies of unknown etiology

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%) (Hayashi et al. J Hum Genet 2011; Uehara et al. J Hum Genet. 2016). Next, 105 cases previously negative for pathogenic CNVs were screened for single nucleotide variants (SNVs) using a 75-gene custom panel. In total, pathogenic variants 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.

2) Copy number variation analysis in early-onset developmental and epileptic encephalopathy

Early-onset developmental and epileptic encephalopathy (DEE) is a group of devastating disorders that appear during the neonatal and infantile periods. Many cases with unexplained etiology remain and, to date, the association of CNVs with early-onset DEE has seldom been addressed. We performed SNP array analysis for 83 cases previously negative for pathogenic SNVs in 109 genes known or suspected to cause epileptic seizures. Rare CNVs were detected in a total of 12 cases (14.4%), of which three cases (3.6%) involved clearly pathogenic CNVs and nine cases (10.8%) were CNVs of uncertain significance. Our findings indicate rare CNVs are also relevant for the diagnosis of early-onset DEEs (Hirabayashi et al. J Hum Genet. 2019).



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Department of Molecular Genetics

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

Breast cancer is a typical hormone-dependent cancer, which is caused by estrogen-dependent cell growth, and utilizes the increase of survival signal due to DNA damage repair dysfunction and ultimately acquires estrogen-independent growth capacity. We investigated the mechanism of breast carcinogenesis by estrogen, the maintenance mechanism of genome stability by BRCA 1 · 2 and have tried developing a novel treatment for breast cancer targeting DNA damage response. Furthermore, we have analyzed the mechanism of DNA stability regulated by estrogen and BRCA2. Our goal is to reveal the functions of molecules associated with breast cancer and to develop novel treatments for breast cancer based on this information.

1. Analysis of disruption mechanism of the breast duct and basement membrane by estrogen

During the progression of breast cancer from ductal carcinoma *in situ* to ductal invasive carcinoma, tumor cells disrupt the breast duct and basement membrane and invade the surrounding tissues, eventually leading to metastasis. However, the molecular mechanisms by which IL-1β and MMP3 are secreted remain unclear. In the present study, we observed that caspase-1 and cleaved gasdermin D (GSDMD) were activated following stimulation with E2. Cleaved GSDMD reportedly induces cell membrane pore formation and contributes to the release of mature cytokines such as IL-1β, which precedes inflammatory pyroptotic cell death. Using electron microscopy, we showed that E2 induced pyroptotic cell death in MCF10A cells and disrupted the basement membrane over MCF10A cells mammary epithelial acini. Collectively, these findings suggest that E2 induces pyroptotic cell death via the caspase-1–GSDMD pathway and promotes the secretion of IL-1β and MMP3, thereby disrupting the breast duct.

2. The physiological roles of interactions between BRCA2 and the estrogen receptor

Estradiol (E2) acts as a transcriptional factor by binding to the estrogen receptor (ERa), and its target genes include BRCA genes. We constructed a plasmid vector that expresses BRCA2-FLAG in an E2-dependent manner and detected the interaction between BRCA2-FLAG and ERa by mass spectrometry analysis. In this study, we report a more detailed analysis of the interaction between BRCA2 and ERa. To consider the direct binding between BRCA2 and ERa, we performed a FLAG-pull-down assay of BRCA2-FLAG and ERa. As a result, we confirmed BRCA2-FLAG and ERa direct binding. Addition of E2 to the complex of BRCA2-FLAG and ERa did not affect the binding between both proteins. However, addition of E2 to ERa before BRCA2 binding was suppressed the binding between BRCA2-FLAG and ERa in an E2 concentration-dependent manner. This suggests that BRCA2 preferentially binds to ERa compared to E2-ERa complex. The endogenous BRCA2 and ERa colocalization was revealed by confocal immunofluorescence images of E2 treated and untreated MCF-7 cells. Endogenous BRCA2 and ERa colocalization was detected in the nucleus with or without E2. These results suggest that BRCA2 and ERa may directly bind in the nucleus, and now we are examining in detail the mechanism by which BRCA2 controls the transcriptional activity of E2-ERa.

3. Identification of two novel breast cancer loci through large-scale genome-wide association study in the Japanese population.

Genome-wide association studies (GWAS) have successfully identified about 70 genomic loci associated with breast cancer. Owing to the complexity of linkage disequilibrium and environmental exposures in different populations, it is essential to perform regional GWAS for better risk prediction. This study aimed to investigate the genetic architecture and to assess common genetic risk model of breast cancer with 6,669 breast cancer patients and 21,930 female controls in the Japanese population. This GWAS identified 11 genomic loci that surpass genome-wide significance threshold of $P<5.0 \times 10\text{-}8$ with nine previously reported loci and two novel loci that include rs9862599 on 3q13.11 (ALCAM) and rs75286142 on 21q22.12 (CLIC6-RUNX1). Validation study was carried out with 981 breast cancer cases and 1,394 controls from the Aichi Cancer Center. Pathway analyses of GWAS signals identified association of dopamine receptor medicated signaling and protein amino acid deacetylation with breast cancer. Weighted genetic risk score showed that individuals who were categorized in the highest risk group are approximately 3.7 times more likely to develop breast cancer compared to individuals in the lowest risk group. This well-powered GWAS is a representative study to identify SNPs that are associated with breast cancer in the Japanese population.

4. 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. Clinical strategies for most of the acquired chemoresistances have not been established yet.

Therefore, we screened chemical compounds to explore novel factors to sensitize tumor cells combined with PARP inhibitor. We utilized known compounds from Chemical Biology Screening Center (TMDU) for drug-repositioning and performed our original screening to assess cytotoxicity and DNA repair machinery. So far, we found sensitizer A as a representative from several perspective candidates. Interestingly, sensitizer A alone did not show a toxic effect and it showed a synthetic lethality only when combined with PARP inhibitor. In addition, sensitizer A increased DNA double-strand breaks, severe damage to cells, with PARP inhibitor by targeting a repair pathway independent of HR repair. These results indicate that a novel synthetic lethal therapy would be developed to overcome various acquired chemoresistances in HBOC. We will investigate the molecular mechanism in detail and develop it for a clinical application.

Highlight

We presented the use of MCF-10A acini in 3D culture as a breast duct model and demonstrated that 17-β estradiol (E2) caused various deleterious effects, including the destruction of the basement membrane surrounding the acini, the abnormal adhesion between cells, and cell death from apoptosis and pyroptosis and that E2 was bound to GPER in MCF-10A cells and stimulated the secretion of matrix metalloproteinase 3 (MMP-3) and interleukin-1β (IL-1β) via the MAPK signaling pathway. The collapse of the breast duct is believed to lead to the initiation of breast cancer cell invasion, resulting in the release of cancer cells in the breast duct as the cancer progresses. Collectively, our findings revealed an important molecular mechanism underlying the effect of estrogen on the collapse of breast duct (Deng *et al.* 2020). We think that further development of this research could verify the possibility that E2 is involved in breast cancer cell invasion. (Figure)

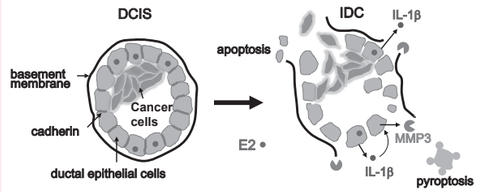

Figure. A schematic model based on our results showing the role of 17-β estradiol (E2) in ductal carcinoma in situ (DCIS) to invasive ductal carcinoma (IDC)
E2 binding to GPER led to cAMP activation and MMP-3 and IL-1β secretion. MMP-3 further degraded cadherin, which adheres to cells that make up the ducts, and laminin in the basement membrane. Moreover, E2 activated caspase-1, which degraded GSDMD to induce pyroptosis. In contrast, IL-1β binds to IL-1R and activates various intracellular signals to promote MMP-3 secretion and induce apoptosis.

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Department of Molecular Epidemiology

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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 regulate human health.

1. The utility of post-test newborn blood spot screening cards for epigenetic association analyses: association between *HIF3A* methylation and birth weight-for-gestational age.

Identification of disease-associated epigenetic markers in early life might be useful for pre-emptive intervention to prevent diseases. Epigenome-wide association analyses using newborn blood spot screening cards are an anticipated field of research in Japan. Here, in this study, post-test dried blood spot (DBS) samples were anonymized, with only three attributes of gender, gestational age, and birth weight identified. We isolated DNA from DBS (n = 300) archived for more than 3 years. The median DNA yield (ng) per individual was 429 (interquartile range 300–565). In a model epigenetic analysis, we conducted a confirmative study on the known association between birth weight and hypoxia-inducible factor 3A (*HIF3A*) gene methylation. DNA methylation levels and cis-acting SNP genotypes (rs8102595 and rs3826795) were measured using EpiTYPER and Taqman assays, respectively. *HIF3A* methylation was positively associated with birth weight-for-gestational age centile (p = 0.021). While *HIF3A* methylation was associated with cis-genotypes (rs8102595, p = 2.08E-13; rs3826795, p = 3.63E-09), the association with birth weight centile was retained after adjusting for cis-genotypes (p = 0.029). Thus, we successfully reproduced the results reported previously by others, and demonstrated the usefulness of archived DBS in secondary use for epigenetic association analyses.

2. 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). In addition, we have started two new projects. One is "Retrospective trajectory analyses of fetal growth and maternal gestational weight gain", and the other is "Regulation of fetal growth and maternal pregnancy adaptation via miRNA-mediated feto-maternal communication".

3. Association Study of Long Non-Coding RNA HOTAIR rs920778 Polymorphism With the Risk of Cancer in an Elderly Japanese Population

The HOTAIR gene encodes a long noncoding RNA (lncRNA), which functions in development and tumorigenesis. A single nucleotide polymorphism (SNP) rs920778 in the HOTAIR gene, has been recurrently studied for susceptibility to many cancers including oesophageal cancer, gastric cancer, lung cancer, and hepatocellular carcinoma. Most of these studies were conducted in Chinese populations, and a few in Turkish, Iranian, and Portuguese populations. They mostly give rise to controversial results. It still remains largely unknown whether the cancer risk is conferred in a Japanese population. Here, we established an association study on the representative SNP rs920778, to examine its contribution to the presence of cancer in consecutive autopsy cases in the JG-SNP database. A total of 1373 subjects (mean age 80) including 827 cancer positive and 546 cancer negative subjects were analyzed. As a result, the occurrence of overall cancer was not associated with the rs920778 polymorphism ($p > 0.05$). For each cancer type, we did not find association except for lung cancer ($p = 0.04$) which was more likely a by-chance association after multiple testing. Our findings imply that rs920778 polymorphism does not affect total cancer presence and the effect on specific cancer types is also weak in the Japanese population.

Association between rs1229984 in ADH1B and Cancer Prevalence in a Japanese Population

Alcohol consumption is an established risk factor for cancer, but little is known regarding the effect of genetic polymorphisms in alcohol metabolism genes on alcohol-related cancer risk in the Japanese population. Associations between the ADH1B gene (alcohol dehydrogenase 1B), single nucleotide polymorphism (SNP) rs1229984 and cancer have been extensively studied yet evidence is inconsistent. This population-based case-control study primarily aimed to clarify any association between SNP rs1229984 in both overall and specific cancer risk in a Japanese population. The functional non-synonymous SNP rs1229984 (Arg48His) was genotyped using DNA samples from 1359 consecutive autopsy cases registered in the Japanese Single Nucleotide Polymorphisms for Geriatric Research (JG-SNP) database. Medical and pathological record data from this database were used to categorise cases and controls. Results included 1359 participants, 816 cases and 543 controls. Multinomial logistic regression analyses showed no significant association between rs1229984 presence and overall cancer risk in both dominant and recessive genetic inheritance models [Arg/Arg+Arg/His vs.His/His: adjusted OR=0.66 (95% CI = 0.39-1.13, p=0.129), Arg/Arg vs Arg/His+His/His: OR=0.95 (95% CI =0.75-1.20, p=0.657)]. However, results showed those homozygous for rs1229984 (genotype His/His) were at significantly decreased odds of lung cancer than other genotypes [recessive model: OR=0.64 (95% CI =0.44-0.93, p=0.020]. In conclusion, there was no significant association between rs1229984 and odds of overall or specific cancers except in lung cancer where His/His genotype decreased odds. To the best of our knowledge, the association between His/His and decreased odds of lung cancer is a novel finding. These findings require further validation in larger studies.

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8. 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 2020*** *in press*

Department of Genomic Function and Diversity

Professor　Yuta Kochi

Research objectives

Complex diseases such as immunological diseases, metabolic diseases and cancer diseases 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 eQTL 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, some of eQTL effects are observed in more cell-specific manners or only in stimulated cells, suggesting further analysis is needed. Moreover, because some of those eQTL effects are observed on distal genes from the eQTL variants (> 100kbp away), additional epigenomic data that uncover 3D structure of genome, such as those obtained by Hi-C, may clarify the regulatory mechanism of eQTL variants.

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.

Furthermore, the contribution of rare variants that GWAS have not targeted is becoming apparent. Some of these rare variants are involved in diseases by qualitatively altering protein functions with amino acid changes. In fact, by resequencing of 98 GWAS genes in rheumatoid arthritis, we revealed accumulation of rare variants in the pseudo-kinase domain of the *TYK2* gene to protect against disease in healthy subjects. Analysis using cell lines revealed that these rare variants attenuated inflammatory cytokine signaling such as IFN-γ, indicating that drug targeting the TYK2 psuedo-kinase domain could be effective.

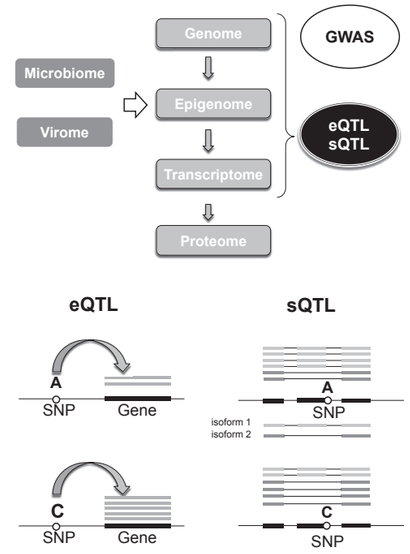
3. Analysis of functional genetic variants and disease mechanism by systems approaches

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.

Highlight

Disease and omics analysis

With the advent of next-generation sequencers, omics data including genome, epigenome, and transcriptome are increasingly deposited in the public databases for disease research. Among them, our main focus is on eQTL and sQTL analysis.



Personnel change

Joined : Asako Yuasa (Secretary), Kensuke Yamaguchi (Collaborative researcher), Kyoko Kobayashi (Collaborative researcher), Mai Abe(Collaborative researcher)

Publications

Original articles

1. Otani T, Noma H, Sugasawa S, Kuchiba A, Goto A, Yamaji T, Kochi Y, Iwasaki M, Matsui S, Tsunoda T. Exploring predictive biomarkers from clinical genome-wide association studies via multidimensional hierarchical mixture models. *Eur J Hum Genet*. 2019 27(1):140-9.
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Review articles

1. Okada Y, Eyre S, Suzuki A, Kochi Y, Yamamoto K. Genetics of rheumatoid arthritis: 2018 status. *Ann Rheum Dis*. 2019 78(4):446-53.
2. Genomics and precision medicine in autoimmune diseases Precision Medicine (in Japanese) 2019 2(1): 10-13
3. Genetics of systemic sclerosis and inflammatory myositis Rheumatology (in Japanese) 2019 61(2): 116-121
4. Research of autoimmune diseases through eQTL studies. Inflammation & immunology (in Japanese) 2019 27(5):398-401

Department of Epigenetics

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Introduction of Department of Epigenetics

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

Research subjects

1. Analysis of genomic imprinting diseases (Highlight)

Paternal and maternal duplication of human chromosome 14 cause genomic imprinting diseases, Kagami-Ogata syndrome and Temple syndrome, respectively. The former is a designated intractable disease in Japan. It is a severe genomic imprinting disease that leads bell-shaped thorax, neonatal lethality associated with respiratory problem. We have recently identified *PEG11/RTL1* is a major responsible gene for these two syndromes as an essential muscle genes in fetal and neonatal periods by analyses of two mouse models (under revision). We are now focusing on the development of new therapy for Kagami-Ogata syndrome by regulating expression of *PEG11/RTL1* using antisense RNAs and siRNAs.

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

We have been carrying out a comprehensive analysis on biological functions of LTR retrotransposon-derived 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 *Sirh11/Zcchc16* is an important brain gene. We are now focusing on *Sirh3/Ldoc1l* (submitted), *Sirh8/Rgag4* and *Sirh4-6/Rtl8c-a* that are also important in brain functions.

3. New method for generation of heart-origanoid

We have developed a new method for generating heart-organoid (HO) from mouse ES cells (under revision) as well as human iPS 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. New method of analyzing DNA methylation status in genomes

We developed a new sequencing method, EnIGMA (Enzyme-assisted Identifidation of Genome Modification Assay) that distinguishes 5-methylcytosine (5mC) and 5-hydroxylmethylcytosines (5hmC) in single DNA fragments. As 5mC and 5hmC may play different roles in gene regulation, we try to expand this method to the genome-wide analysis.

Highlight

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 related symptoms, such as muscle hypotonia, feeding difficulty/poor sucking function in early childhood in the former, in contrast to neonatal lethality due to respiratory problems associated with a bell-shaped thorax and abdominal wall hernia in the latter (Fig. 1). We have previously demonstrated that mouse *Peg11/Rtl1* is one of the major genes responsible for the placental abnormalities characteristic of these two syndromes. However, the role of *Peg11/Rtl1* in the respiratory failure and other muscular problems observed in mouse neonates remains unknown. Our two mouse models, *Peg11/Rtl1* Pat-KO (loss of *Peg11/Rtl1*) and Mat-KO (overproduction of *Peg11/Rtl1*) mice exhibit similar abnormalities to the Temple and Kagami-Ogata syndromes, respectively. 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). This is the first demonstration that an LTR retrotransposon-derived *Peg11/Rtl1* plays an important role in fetal and neonatal muscle development, strongly suggesting a critical involvement of *PEG11/RTL1* in these diseases. We have also demonstrated that the Peg11/Rtl1 protein localizes around the Z-disc in muscle fiber also interacting the Desmin that is essential for skeletal muscle stability and force generation (Fig. 3). 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 (under revision)).

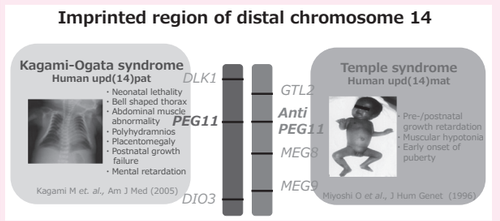

Figure 1. Kagami-Ogata syndrome and Temple syndrome.
(Left) The features of Kagami-Ogata syndrome (Upd(14)pat) and (right) those of Temple syndrome (Upd(14)mat). Both exhibit muscle-related defects.

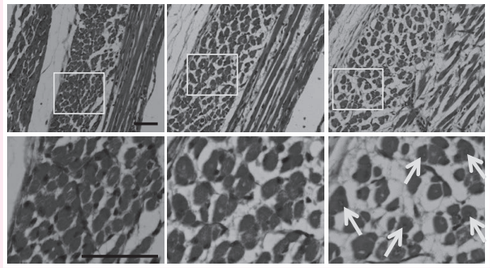

Figure 2. Muscle abnormalities in loss and overprocdution of *Peg11/Rtl1*.
(Upper columns): HE staining of the neonatal intercostal muscle. (lower column): a more highly magnified view of the yellow boxes in the upper column. (left) *Peg11/Rtl1* KO mouse (Pat-KO) (center) wild type mouse, (right) *Peg11/Rtl1* overproduction mouse (Mat-KO). Scale bars, 1 mm (upper columns) and 50 μm (lower columns), respectively.

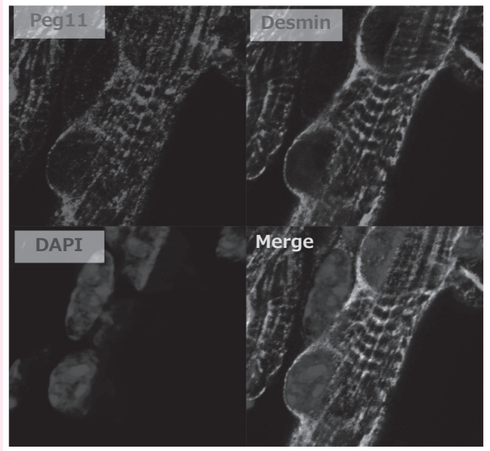

Figure 3. Expression of Peg11/Rtl1 protein in muscle fibers.
Immunofluorescence staining of Peg11/Rtl1 protein in the neonatal abdominal muscles from Mat-KO mice. Long axis views of the muscle fibers. Co-immunostaining with Peg11/Rtl1 (red; arrowheads), Desmin (green; arrows) and DAPI (blue), and their merged images. Scale bars, 20 μm. The neonates were not fixed before being embedded in the OCT compound.

Publications

Original papers and reviews

1. Kaneko-Ishino T and Ishino F. Evolution of viviparaity in mammals: what genomic imprinting tells us about mammalian placental evolution. Reprod Fert Dev (in press) doi: 10.1071/RD18127
2. Kaneko-Ishino T and Ishino F. Cooperation and Competition in Mammalian Evolution -Gene Domestication from LTR Retrotransposons- *In* Evolution, Origin of Life, Concepts and Methods, Chapter 15 (Ed. Pontarotti P), Springer Nature, pp.317-333 (2019).

Department of Medical Science Mathematics

Professor	Tatsuhiko Tsunoda
Junior Associate Professor	Fuyuki Miya
Assistant Professor	Jo Nishino
Secretary	Yumi Nakamura

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. Genomic data analysis methodology with deep learning

Recently, we have developed a new schema to analyze non-image data, e.g. gene-expression profiles, with deep neural network by converting the data into an image-like form **[1]**. Since omic data are non-image data, to fully utilize the ability of deep learning, we convert them to image-like form. To accurately classify samples to subtypes with omic data for disease diagnosis and prediction, we developed the DeepInsight method which consists of three steps: reallocation of variables, feature extraction, and classification. Applying this method to several tasks, e.g. cancer classification, we found that it can perform much better than traditional machine learning methods. This technique is generally applicable for extracting features to classify samples in biomedicine.

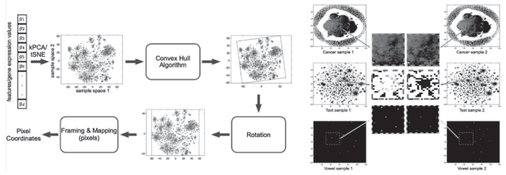

Figure 1 Converting non-image data, e.g. omic data, to image data. The method (left) and application to real data (right).

2. New network-analysis methodologies for identifying disease modules, and drug response and toxicity.

We have developed original methodologies for network topology analysis for identifying key molecules for disease and drug-response and constructing their prediction models, and a highly-accurate model for predicting drug-toxicity by using machine learning to combine features from omic and physicochemical properties. We participated in the DREAM challenge competitions, to which we applied our methodologies, and won a best-performer award, and published the results **[2-4]**.

3. Dementia subtype classification and risk prediction

There are roughly three subclasses of dementia, and it is considered important to properly classify and diagnose them for treatment. We developed models to accurately classify them and predict their risk for individuals using miRNA expression data with semi-supervised and machine learning methods **[5,6]**.

4. Single cell clustering method for small sample set

Although researchers have been applying clustering techniques to single-cell RNA-Seq data, small sample size is one of big issues. To overcome this, we developed original method with feature clustering and selection **[7]**.

5. 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 **[8]**.

6. GWAS – biomarker identification, prediction, and textbook

Genome-wide association studies (GWAS) are expected to be applied for exploring biomarkers and for predicting disease risk in the near future. We developed such methodologies, and published a text book describing GWAS history, method, results, and future perspectives **[9-12]**.

7. Post-translational modification prediction

Recently, we have developed prediction models for post-translational amino-acid modifications and protein structures using various physicochemical and sequence properties **[13]-[19]**,

8. Recognizing and predicting brain wave classification

We developed methodologies for recognizing and predicting brain wave classification using frequency spectrum analysis and deep learning, which achieved high accuracy **[20,21]**.

In addition to the above, we are now collaborating with several clinical trial groups for cancer immunology to identify biomarkers and construct drug-efficacy prediction models.

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21. Kumar S, Sharma A, Tsunoda T. Subject-Specific-Frequency-Band for Motor Imagery EEG Signal Recognition Based on Common Spatial Spectral Pattern. In: Nayak A., Sharma A. (eds) ***PRICAI 2019: Trends in Artificial Intelligence***. *PRICAI 2019. Lecture Notes in Computer Science*, vol 11671, 712-722, Springer, Cham, 2019.

Frontier Research Unit
Laboratory of Gene Expression

Associate Professor Hidehito KUROYANAGI

Post-transcriptional regulation is an important layer for gene expression regulation. Based on recent transcriptome analysis, 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 determines the cell-type specific pre-mRNA processing patterns and elucidate molecular basis for pathogenesis of genetic diseases caused by defects in the post-transcriptional regulation of gene expression.

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

We have developed a transgenic fluorescence alternative splicing reporter system that enables visualization of alternative pre-mRNA processing patterns *in vivo* (Nat Meth, 2006; Nat Protoc, 2010). With this reporter system, we have visualized spatiotemporal profiles of 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). We have recently reported that alternative splicing of a tropomyosin gene is differentially regulated in the head muscles and confers a specific function (Mol Biol Cell, 2018; Cytoskeleton, 2018). Through these studies, we now realize that molecular mechanisms for the post-transcriptional gene regulation are conserved throughout metazoan evolution (WIREs RNA, 2017; WormBook, in press).

Alternative Splicing Regulator RBM20 in Dilated Cardiomyopathy.

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 have recently 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, was essential for nuclear localization, which allows RBM20 to interact with its target pre-mRNAs (Sci Rep, 2018). We generated an *Rbm20*^{S637A} knock-in mouse, mimicking an un-phosphorylatable mutation found in a well-studied case of DCM, and confirmed that the residue is critical for the splicing regulation by RBM20 in the heart (Sci Rep, 2018) (Figure). Further study of the knock-in mouse will lead to better understanding of the DCM pathogenesis and developing therapeutics (Front Mol Biosci, 2018). We are also collaborating with a group in Mayo Clinic, USA on a pig model of DCM caused by another missense mutation in the RSRSP stretch (Nat Med, in press).

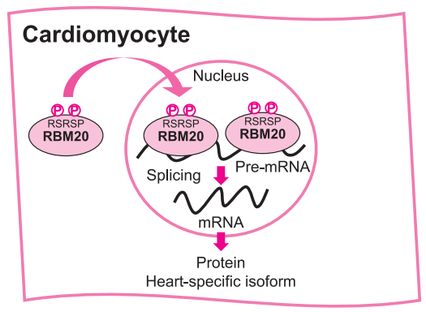


Figure. Phosphorylation of the RSRSP stretch is critical for nuclear localization and functions of the RBM20 protein

Publications

Original Articles

1. 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, Alex 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. A ribonucleoprotein-granule pathway to heart failure in human RBM20 cardiomyopathy gene-edited pigs. **Nature Medicine**, in press.

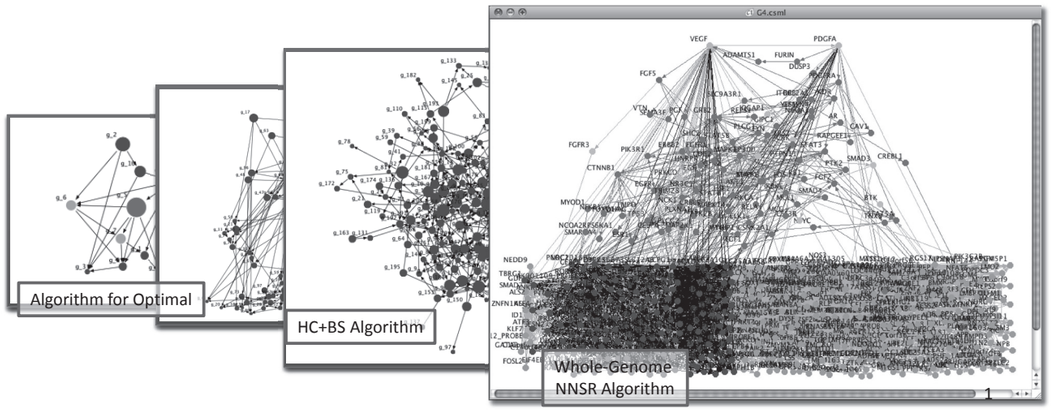
Review Article

1. Joshua A. Arribere, Hidehito Kuroyanagi and Heather A. Hundley. mRNA editing, processing and quality control in *Caenorhabditis elegans*. **WormBook**, in press.

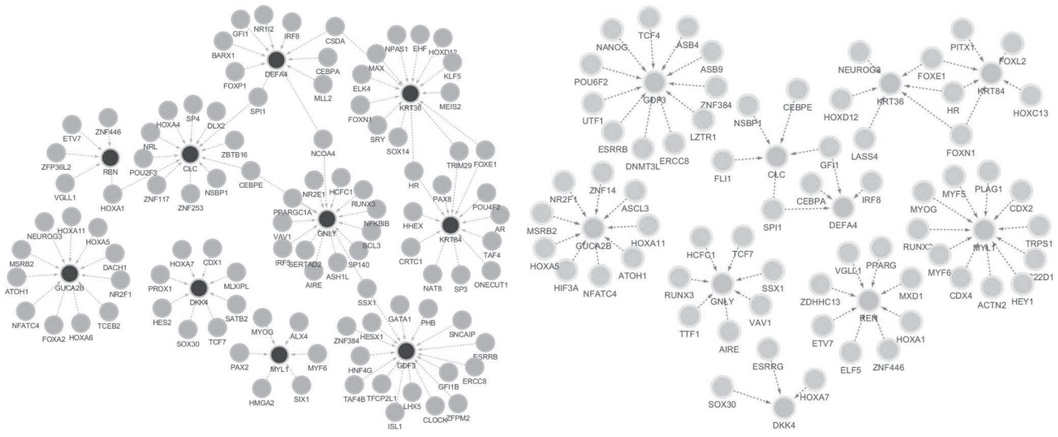
Laboratory for Integrated Research Projects on Intractable Diseases
Advanced Technology Laboratories

Systems Biology for Intractable Diseases

It is getting clearer that pathogenesis of intractable disease is a state that deviates from an integrated systems control in the 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. 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.



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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3. The ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium. Pan-cancer analysis of whole genomes. ***Nature***. 2020 Feb;578(7793):82-93. doi: 10.1038/s41586-020-1969-6. Epub 2020 Feb 5.
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Laboratory for Integrated Research Projects on Intractable Diseases

IBD project3 Laboratory for Integrated Research Projects on Intractable Diseases

Professor Shigeomi SHIMIZU
Toshiaki OHTEKI

Summary

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

Research Outcome

1. Epithelial regeneration mechanisms in the intestine: Disruption of the intestinal epithelial barrier function provokes an excessive immune response through the invasion of intestinal commensal bacteria into the host. As this excessive immune response induces the pathogenesis of IBD, elucidation of the mechanism of intestinal epithelial regeneration is important. 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, the balance of YAP/Wnt signal is important for the epithelial regeneration ability of the origin cells, and one of the tetraspanin family members is a useful marker to identify the fraction including the cell of origin (in revision). We also found that chronic IFN signaling reduces the stemness of ISCs using DSS colitis and 5-FU enteritis models (in revision).

2. Involvement of autophagy in IBD：In order to elucidate the relationship between autophagy and IBD, we generated various types of intestine-specific autophagy knockout mice. We also searched for small compounds that improve IBD via autophagy induction. To this end, we first developed a new method to monitor autophagy dynamics using small cell-permeable fluorescent probes, namely, DAPRed, DALGreen, and DAPGreen. Using these probes, we were able to easily detect autophagy flux as well as the generation kinetics of omegasomes, autophagosomes, and autolysosomes (submission). Then, we performed high-throughput screening of a chemical library, and identified 120 small compounds and 31 natural products that induce autophagy. Among them, we identified one natural products that improve IBD (in revision).

Publications

1. Wang S, Adachi S, Kong L, Watanabe D, Nakanishi Y, Ohteki T, Hoshi N, and Kodama Y. Role of eosinophil in a murine model of inflammatory bowel disease. Biochem **Biophys Res Commun** 2019; 511: 99-104.
2. Tezuka H, and Ohteki T. Regulation of IgA production by intestinal dendritic cells and related cells. **Front Immunol** 2019; 10: 1891.
3. Sugimoto Y, Murohashi M, Arakawa S, Honda S, Shimizu S. Prediction of intracellular targets of a small compound by analyzing peptides presented on MHC class I. **Biochem Biophys Res Commun** 2019; 508: 480-486.
4. Noguchi S, Honda S, Saitoh T, Matsumura H, Nishimura E, Akira S, Shimizu S. Beclin 1 regulates recycling endosome and is required for skin development in mice. **Communications Biology** 2019; 2, Article No. 37.

Research Project on Heart Diseases

Project Leader, Gene Expression: Hidehito KUROYANAGI
Bio-informational Pharmacology: Tetsushi FURUKAWA, Kensuke IHARA
Molecular Neuroscience: Yuichi HIRAOKA
Cardiovascular Medicine, Graduate School of Medical and Dental Sciences: Tetsuo SASANO

Alternative Splicing Regulator RBM20 in Dilated Cardiomyopathy.

Dilated cardiomyopathy (DCM) is a heart disease characterized by left ventricular dilatation and systolic dysfunction. DCM can be caused by mutations in sarcomere protein genes including *TTN*. Pre-mRNA splicing of the *TTN* gene is developmentally regulated and varies between cardiac and skeletal muscles. An RNA-binding protein RBM20 is the major regulator of the heart-specific alternative splicing of *TTN*. Mutations in *RBM20* are also known to cause autosomal-dominant familial DCM and most of the *RBM20* missense mutations were mapped to an RSRSP stretch. We constructed a fluorescence reporter mini-gene for *TTN* to analyze the heart-specific splicing regulation of RBM20. We revealed that phosphorylation of the RSRSP stretch on the two serine residues is critical for nuclear localization of RBM20. Furthermore, we identified an S634W missense mutation within the RSRSP stretch in a family of DCM patients, which also affected nuclear localization of RBM20. $Rbm20^{S637A}$ knock-in mouse showed a remarkable effect on titin isoform expression. Our study unveiled the function of the RSRSP stretch as a critical part of the nuclear localization signal and offers the $Rbm20^{S637A}$ mouse as a model for DCM (Sci Rep, 8: 8970, 2018; Front Mol Biosci, 5: 105, 2018).

Publications

[Original papers]
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, Alex 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. A ribonucleoprotein-granule pathway to heart failure in human RBM20 cardiomyopathy gene-edited pigs. **Nature Medicine**, in press.

Hypoxic Breast Cancer Project

Principal Researcher Koh Nakayama, Ph.D.
Project collaborators Hiroshi Shibuya Ph.D.
Yoshio Miki, M.D., Ph.D.
Fumitoshi Ishino, Ph.D.

Research Subject

Tumor microenvironment is often hypoxic, and induces malignant transformation of cancer cells. Breast cancer patients are increasing every year in women worldwide. Thus, it is important to understand how hypoxic environment affects breast cancers. 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 regulatory mechanism of gene expression in breast cancers which is mediated by epigenetics. We currently analyze DNA demethylating enzyme TETs. TETs mediate gene expression by altering the methylation status in cells. Hypoxic regions are formed in breast tumors, and DNA methylation is increased in breast cancers. However, it remains unclear if hypoxia has any impact on the DNA methylation status of breast cancers. We will address this question by combining approaches based on hypoxic and epigenetic studies (Figure). Our final goal is to establish a new technology to detect breast cancers at an early stage by using the molecules identified in this project.

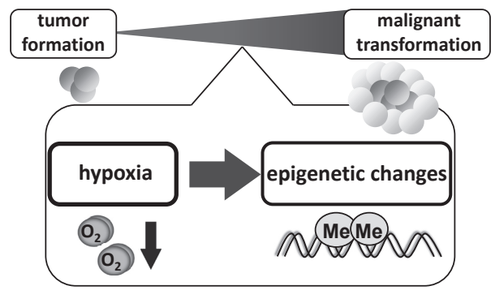


Figure. Scheme of Hypoxic Breast Cancer project

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 node, resulting in their poor prognosis. In addition, surgical treatment against these types of cancer causes the deterioration in the quality of life due to the change in the facial appearance and difficulty in meal intake. Despite the accumulating knowledge on HNSCC and ESCC, optimal interventions to overcome-those insufficient conditions have not been developed so far. In this project, our aim is the establishment of precision medicine for HNSCC and ESCC through omics data analysis using information of genomics, epigenomics, transcriptomics, proteomics, metabolomics and phenomics.

We have constructed a network for collecting and analyzing clinical samples through a cooperation between the medical and dental hospitals and the Bioresource Research Center (BRC) of TMDU. Over 800 clinical samples have been collected as of Dec. 2019 (HNSCC: 569 samples, ESCC: 255 samples).

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 the development. Thus, we aimed to find novel anti-cancer drugs through a cell growth screening assay of a 766-drug library, approved by the Food and Drug Administration, in HOC313-LM cells, a highly metastatic oral squamous cell carcinoma (OSCC) subline established from a parent line, HOC313. As a result, pitavastatin emerged as a candidate anti-cancer drug. Pitavastatin is known to inhibit the mevalonate pathway through the block of HMGCR and is applied to hypercholesterolemia in the clinical setting, but not to cancer so far. We confirmed that pitavastatin induced the cell death through the downregulation of AKT and ERK signals in OSCC and ESCC cell lines. We next performed an *in vivo* tumor growth assay by adding pitavastatin into chicken embryo and mouse. Pitavastatin could reduce the tumor growth *in ovo/in vivo* as well as *in vitro*. Moreover, the effect of pitavastatin was canceled by adding mevalonate or geranylgeranyl pyrophosphate (GGPP), two metabolites of the mevalonate pathway. In addition, we have identified an optimal biomarker to stratify an effective responder for treatment of pitavastatin in OSCC and ESCC. Taken together, our findings suggest that pitavastatin might be a potential anti-cancer drug in OSCC and ESCC.

DOHaD research towards preventive and preemptive approach against chronic intractable disease

Project Leader　Noriko Sato
Collaborators　Naoyuki Miyasaka, Tomonori Ishikawa, Ayako Fudono (Comprehensive Reproductive Medicine), Sumio Sugano, Chihiro Imai (Molecular Epidemiology)

Summary

Multiple lines of evidence from epidemiological observations have implicated that the quality of fetal development is linked to risks of noncommunicable diseases later in life. Developmental Origin of Health and Disease (DOHaD) was conceptualized, which means that the developing conditions *in utero* or in 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 started two new projects. One is "Retrospective trajectory analyses of fetal growth and maternal gestational weight gain", and the other is "Regulation of fetal growth and maternal adaptation to pregnancy via miRNA-mediated feto-maternal communication".

Research Outcome

Heterogeneity in fetal growth velocity

Fetal growth quality is associated with susceptibility to non-communicable diseases. fetal size has been conventionally assessed using the averaged growth chart, but fetal growth velocity has recently been attracting attention as another important aspect of fetal development. Since fetal growth velocity may reflect fetal response to various conditions during the developmental process within the maternal constraint, it is reasonable to imagine that there might exist a physiological diversity in growth velocity patterns over time, which has never been explored. We conducted a retrospective cohort study designed to evaluate the heterogeneity of fetal growth velocity in singleton pregnancies in the Japanese population. We leveraged the high frequency of prenatal checkup to collect large numbers of ultrasound measurements of every fetus (n = 801) and computationally analyzed individual changes in growth per week. Latent class trajectory analysis identified three distinct velocity patterns. The variation in growth velocity appeared in the third trimester and corresponded to the differences in neonatal size. this heterogeneity was not simply explained by maternal factors and fetal sex, although those factors had time-varying effects on fetal size. Our findings regarding the heterogeneity in fetal growth velocity will aid in the comprehensive understanding of fetal development quality.

Publications

[Original Paper]

1. Pavethynath S, Imai C, Jin X, Hichiwa N, Takimoto H, Okamitsu M, Tarui I, Aoyama T, Yago S, Fudono A, Muramatsu M, Miyasaka N, Sato N. Metabolic and Immunological Shifts during Mid-to-Late Gestation Influence Maternal Blood Methylation of *CPT1A* and *SREBF1*. ***Int J Mol Sci.*** 2019;20(5). pii: E1066.
2. Kyaw TZ, Yamaguchi S, Imai C, Uematsu M, Sato N. The utility of post-test newborn blood spot screening cards for epigenetic association analyses: association between *HIF3A* methylation and birth weight-for-gestational age. ***J Hum Genet.*** 2019;64(8):795-801
3. Sato N, Miyasaka N. Heterogeneity in fetal growth velocity. ***Sci Rep.*** 2019;9(1):11304.
4. Sato N, Miyasaka N. Stratified analysis of the correlation between gestational weight gain and birth weight for gestational age: a retrospective single-center cohort study in Japan. ***BMC Pregnancy Childbirth.*** 2019;19(1):402.

Project for Hereditary Osteolysis

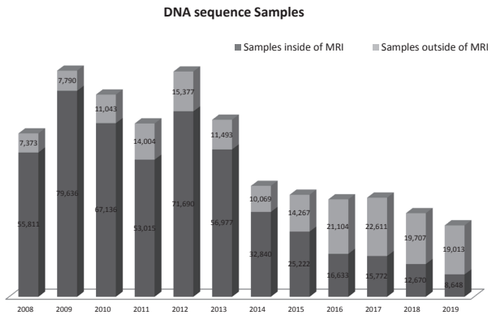
Yoichi Ezura

To develop a therapeutic agent effective for treating hereditary osteolysis, we search for the chemicals that are effective for improving the symptoms of disease model, i.e., the mice conditionally lacking profilin 1 (Pfn1) specifically in osteoclasts. Especially, the agents that inhibit cell motility will be evaluated by comparing to the effects of bisphosphonate and cathepsin K inhibitor that are already known to be potent therapeutic agents for osteoporosis. This year, we first tested the effect of CK-666, which inhibits Arp2/3, in osteoclast culture, focusing on its inhibitory effects on migration and bone resorption. Also in vivo effects by intermittent administration were tested in 8-week-old mice on bone mass change in comparison to that of alendronate. By the way, a PFN1 mutation was reported from abroad as a new causal mutation in one family with a diagnosis of "Paget's disease of bone" that develops a systemic osteolytic lesion but no mutation in the SQSTM1 gene was identified. Therefore, the significance of the Pfn1-cKO mouse that we use as a model system has been further clarified.

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 Center of the University.



Followings are the achievements in 2019.

1. Sequencing analyses

A total of 27,661 samples from 2,824 researchers were sequenced in the year of 2019. Among them 19,013 (69%) 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 16 runs were done in the year of 2019.　Library preparation service for next generation sequencing has been started in 2015 and 280 samples were done in the year of 2019.

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.

3. Introductory seminars

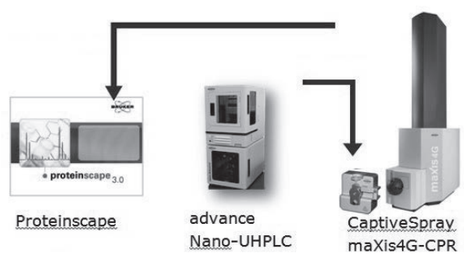
Introductory seminars were done for use of　instruments (2times).

Laboratory of Cytometry and Proteome Research

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

For proteome analysis, we have a 2D-electrophoretic system, LC-MSMS systems and a Biacore system, MicrocalorimetryTC200, Eppendorf InjectMan NI2, Leica M165FC in this laboratory. We can accept the consignment analysis of proteins with 2D-electrophoresis and the mass spectrometry by request of researchers in this university. In addition, we can provide technical advices on cytometry and proteome researchers who wish to start their own research.

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



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.

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 and Laser Capture Microdissection　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…6/3 (Carl Zeiss)

Laser Capture Microdissection…5/13(Leica)

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 four Professors and two Associate Professors in the Institute, and the services are provided by one Technical Staff and one Technical Assistant.

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

The number of overall use cases was 588 in the year of 2019. 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 Faculty of Medicine of Yamanashi university and Eisai Co., Ltd., and provided them with some cell lines. In response to this, we reviewed the off-campus offer price, and the agreement was received to change the price of selling to commercial organizations to individual rates (Effective December 1). 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 a new request from the joint research group between Showa University and the laboratory of our Medical research institute. We also undertake with mycoplasma contamination test (see Fig.1). In this year, inspection requests decreased compared to the previous year. This phenomenon is probably due to the fact that each researcher was alert to mycoplasma contamination during cell line manipulation, and as a result, much of mycoplasma samples were negative and the number of re-requests for testing was small. 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.

In addition, in the same year, we participated in the "Sheard use of university-owned equipment that contributes to drug discovery and medical open innovation" with Tokyo, and accepted support tours for seven venture companies and Mitsui Fudosan Co., Ltd.

In March, we changed our website and worked to disseminate more accurate information on support contents and commission fee.

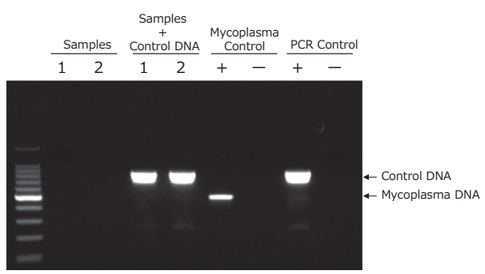


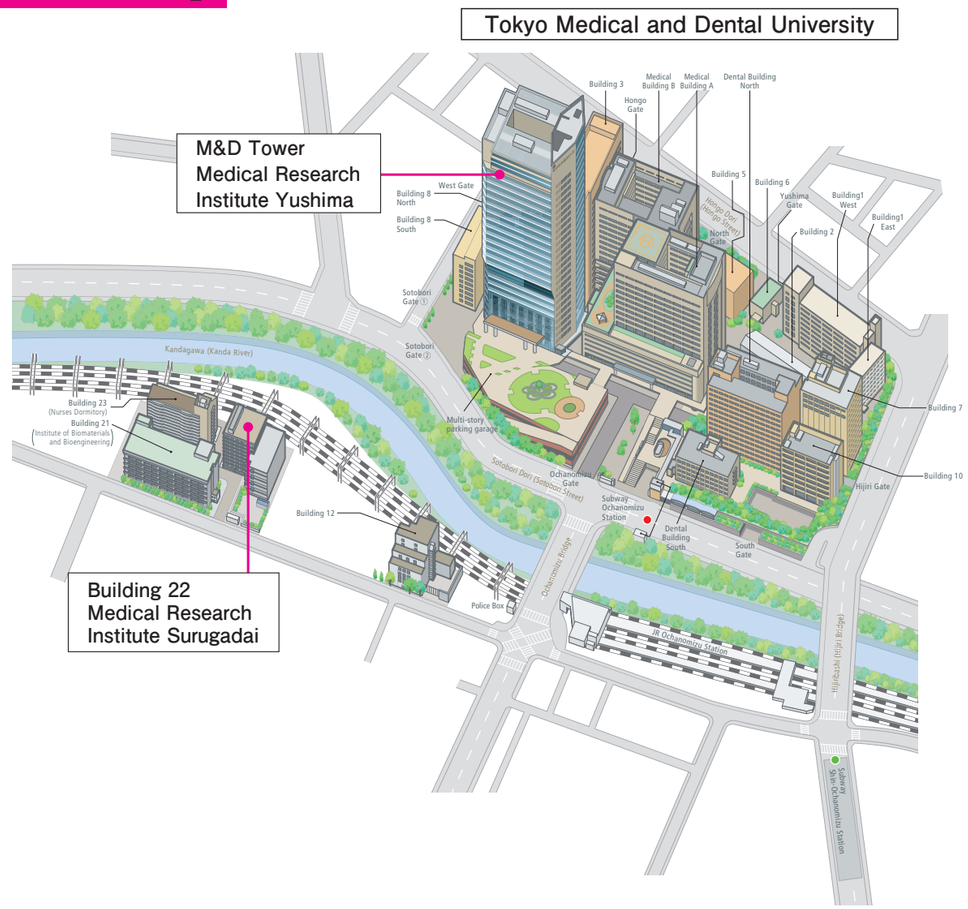
Fig. 1 Mycoplasma Test

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

Laboratory for Structure Analysis

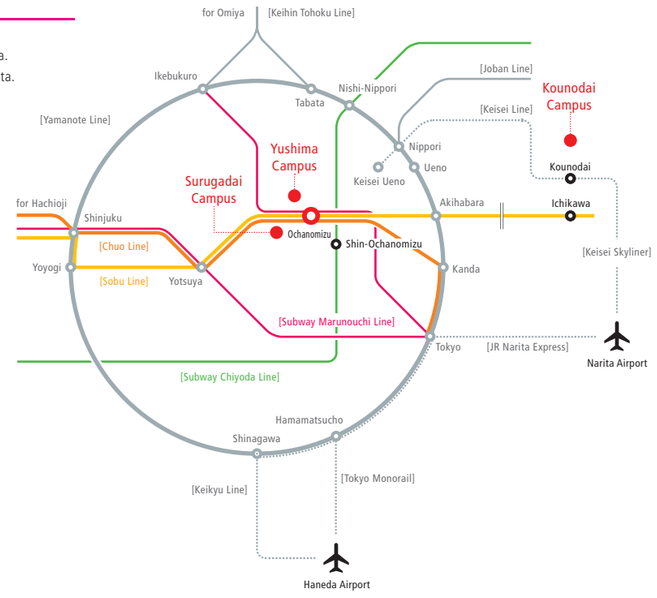
The Laboratory for Structure Analysis is equipped with a high-brilliancy X-ray generator (Rigaku MicroMax007HF) and an imaging plate X-ray detector (Rigaku R-AXIS VII) for the structure determination of biological macromolecules. The laboratory is also equipped with a dynamic light scattering (DLS) instrument (Malvern Zetasizer μV), enabling the measurements of the particle size (thus the oligomeric state and aggregation) of protein molecules. An introductory practical course was held for students of Graduate School. The laboratory joined the Joint Usage/Research Program of the Institute and is open for users from the outside of the university.

Access Map



Nearest Stations

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



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