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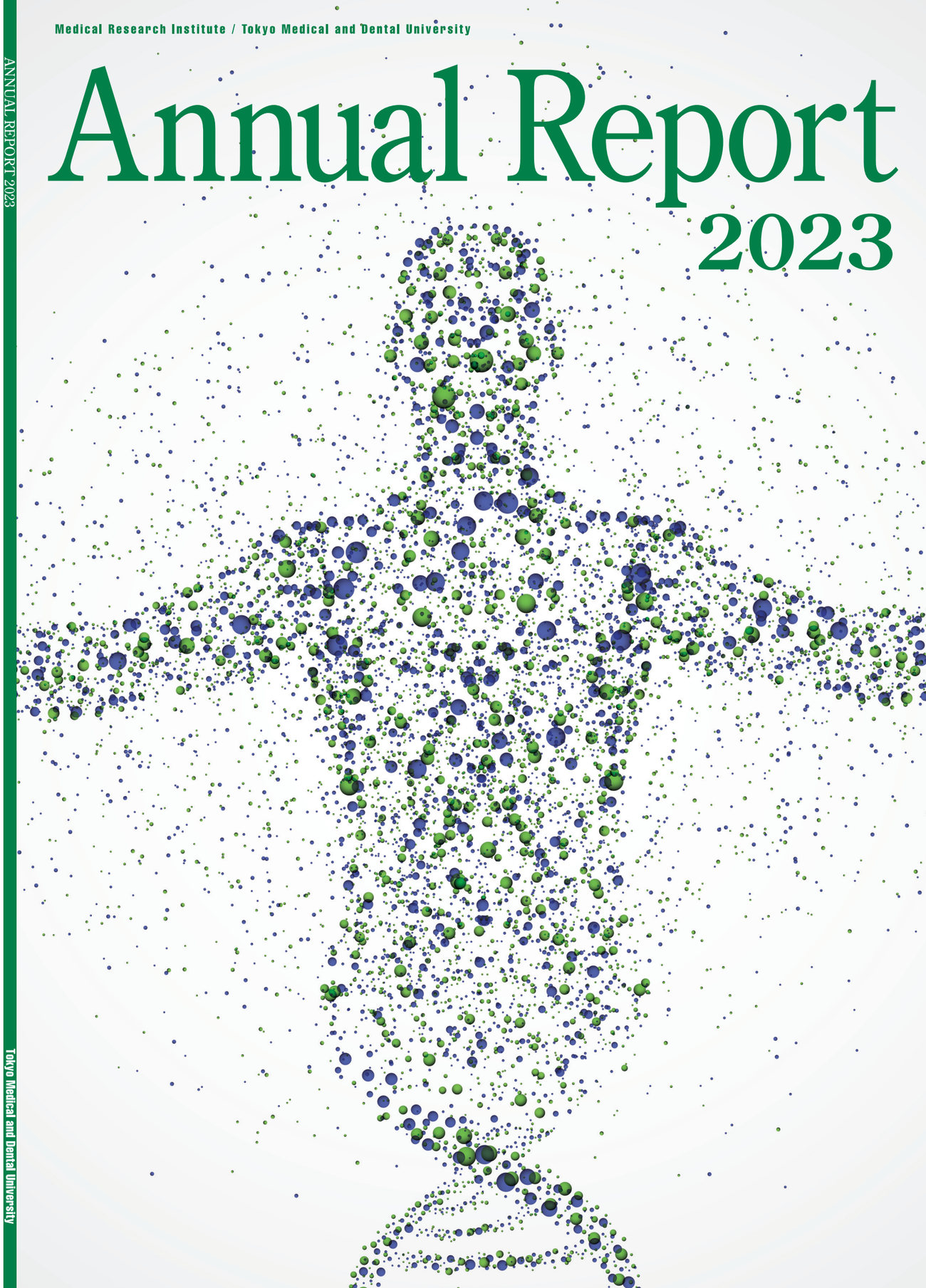
ANNUAL REPORT 2023

Tokyo Medical and Dental University

Medical Research Institute / Tokyo Medical and Dental University

Annual Report 2023



2023

Annual Report

Medical Research Institute

Tokyo Medical and Dental University

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Medical Research Institute

Yushima Area

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

Medical Research Institute

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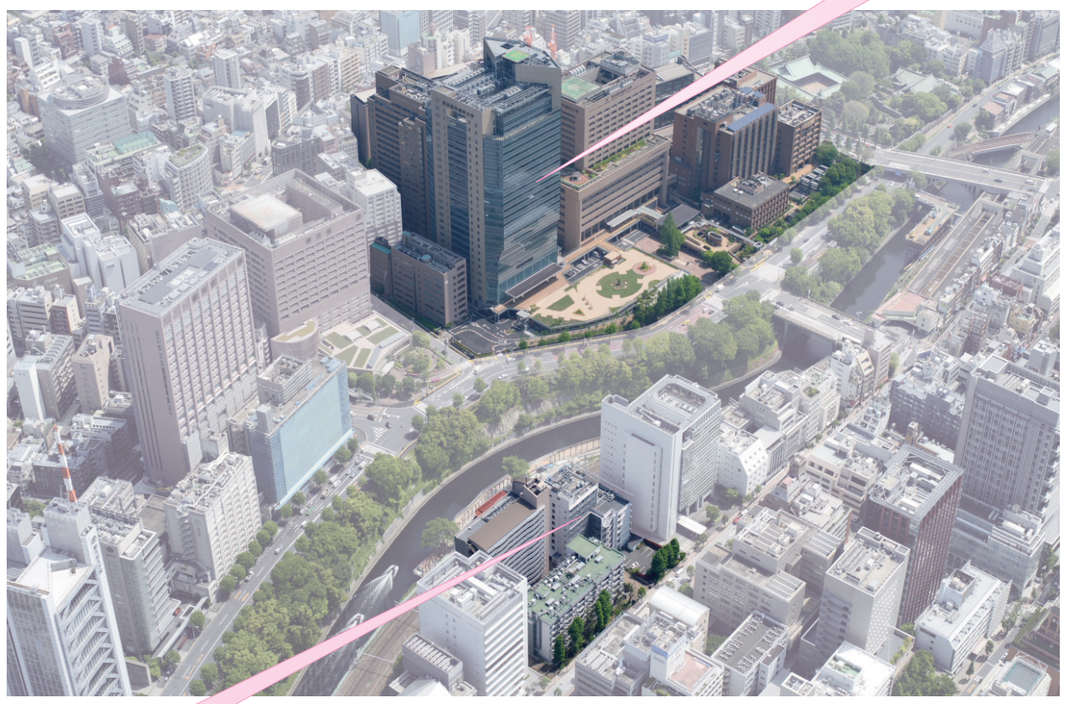

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Medical Research Institute

Department of Precision Health, Laboratory of Genome Editing for Biomedical Research

Medical Research Institute

- Director: Hiroshi Nishina
- Deputy Director: Yuta Kochi
- Faculty Meeting
- Research Divisions
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    - Department of Molecular Cell Biology — Professor — Hiroshi Shibuya
    - Department of Stem Cell Regulation — Professor — Tetsuya Taga
    - Department of Molecular Epidemiology — (under consideration)
    - Department of Homeostatic Medicine — Professor — Fumiko Toyoshima
    - Frontier Research Unit
  - Division of Advanced Pathophysiological Science
    - Department of Biomolecular Pathogenesis — Professor — Noriyuki Matsuda
    - Department of Biodefense Research — Professor — Toshiaki Ohteki
    - Department of Neuropathology — Professor — Hitoshi Okazawa
    - Department of Molecular Neuroscience — Professor — Kohichi Tanaka
    - Department of Pathological Cell Biology — Professor — Shigeomi Shimizu
    - Department of Neuroinflammation and Repair — Professor — Takashi Shichita
    - Department of Molecular Genetics — (under consideration)
    - Frontier Research Unit
  - Division of Biological Data Science
    - Department of Structural Biology — Professor — Nobutoshi Ito
    - Department of Functional Genome Informatics — Professor — Itoshi Nikaido
    - Department of Genomic Function and Diversity — Professor — Yuta Kochi
    - Department of Bio-informational Pharmacology — (under consideration)
    - Department of Molecular Cytogenetics — (under consideration)
    - Department of Computational and Systems Biology — Professor — Teppei Shimamura
    - Department of Advanced Nanomedical Engineering — Professor — Satoshi Uchida
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  - Laboratory of Cytometry and Proteome
  - Laboratory of Genome Editing for Biomedical Research
  - Laboratory of Anatomy and Cell Function
  - Bioresource Laboratory
  - Laboratory for Structure Analysis
  - Stem Cell Laboratory
  - Single-cell Omics Laboratoy
- Administration Office
  - General Affairs Unit

Medical Research Center Initiative for High-Depth Omics

The Medical Research Institute, Tokyo Medical and Dental University, in collaboration with The Medical Institute of Bioregulation, Kyushu University, the Institute of Developmental Biology, Kumamoto University, and the Institute of Advanced Medical Sciences, Tokushima University, has launched Medical Research Center for High Depth Omics in FY2022. In this project, we will conduct high-deep omics research to measure the mechanisms of biological phenomena and disease onset at the single cell and single molecule level with high resolution and high resolution and to integrate these big data.

Project Background

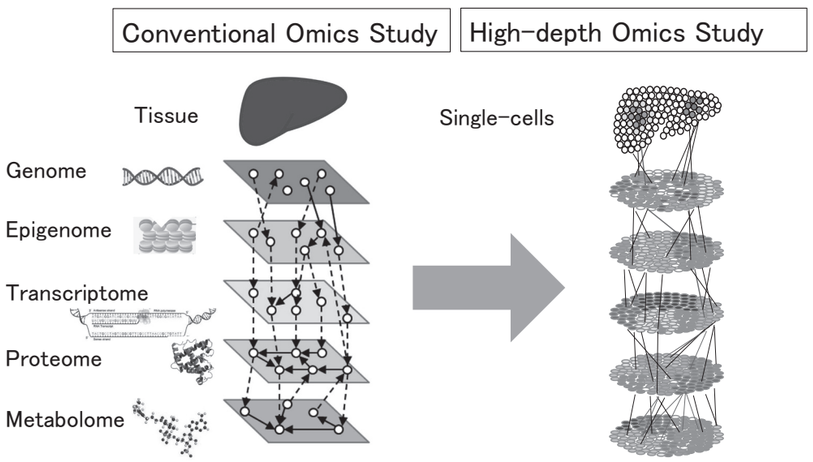
On June 25, 2009, the Medical Research Institute, Tokyo Medical and Dental University, was certified by the Minister of Education, Culture, Sports, Science and Technology as a “Joint Research program for Medical Research.” In 2010, the center was a “Joint Research Program for Medical Research” as the second phase of the joint program for medical research.
We also have promoted joint research with the community of researchers conducting research on diseases by the mission of this center since 2022 as the third phase of the joint program for medical research.

Purpose of the Project

The human body is composed of many cell types, and the correct regulation of their cellular composition and characteristics is essential for healthy development and health maintenance. To identify the cell types in complex organs and tissues and to measure their characteristics, it is necessary to determine what genes are active in the cells and at what levels. To be determined which and how genes are activated in the specific cell, it is necessary to analyze the whole RNAs in the cell comprehensively. This technique is called transcriptome analysis. However, conventional transcriptome analyses are carried out at the organ/tissue level. The method cannot capture changes in the cellular composition and characteristics of the constituent cells of organs/tissues. In this project, we will conduct high-deep omics research to measure the mechanisms of biological phenomena and disease onset at the single-cell and single-molecule level with high resolution and high resolution and to integrate these big data.

A technology that enables high-throughput measurement of the transcriptome in single cells is required to accurately capture all cell types in which organs and tissues are contained and the function of all genes working in the cells. Quartz-Seq2, the world’s highest-performance single-cell transcriptome technology developed by the Medical Research Institute in collaboration with RIKEN, can precisely measure the transcriptome of thousands to tens of thousands of single cells that make up an organ or tissue. This technology is expected to dramatically advance our understanding of the causes of intractable diseases such as cancer and psychiatric disorders and provide information on cell composition and tissue characteristics, which is essential for regenerative and reproductive medicine.

Through the network of the Medical Center of Excellence for Omics Research, the institute will disseminate single-cell omics technologies such as Quartz-Seq2 to the scientific community while integrating data from genomic polymorphism function analysis, lipidomics, and cryo-electron microscopy, which are the strengths of our institute, in order to promote genomic medical research in Japan greatly. We aim to promote significant genomic medical research in Japan.



Activities in FY2022

Establishment of the Single Omics Laboratory in the Medical Research Institute
The Single Cell Omics Laboratory was established as the core laboratory of this project. The Single Cell Omics Laboratory cooperates with the Department of Functional Genome Informatics and the Research Core Center at TMDU. This year, we proceeded with the laboratory’s setup, the equipment’s installation, and the technical staff’s hiring. We established A management committee for the laboratory, and four meetings were held. In addition, four joint research projects were conducted within and two outside the university (one was with a company), 20,000 samples were sequenced, and two papers were published.

Events

The 1st Symposium of the High-Depth Omics Medical Research
Date: November 16-17, 2022
Method: Online
Location: Kumamoto

Division of Visionary Life Science

【Aim and Scope】

The Division of Visionary Life Science aims to pioneer new medicine through research into the fundamental mechanisms of life. Based on this philosophy, we will discover the etiology of intractable diseases, elucidate pathological conditions, and lay the foundation for the development of diagnostic, therapeutic, and preventive methods by developing and utilizing cutting-edge biological samples and methods, including diseased ES/iPS cells, cancer stem cells, organoids, disease model animals, and mass spectrometry techniques. Our division's significant research achievements this year are as follows;

【Medical Chemistry】

- Elucidation of mechanisms for the asymmetrical distribution of phosphatidylserine supported by two distinct pathways by four flippases
- Elucidation of mechanisms of inefficient development of syncytiotrophoblasts in the Atp11a-deficient placenta

【Biochemical Pathophysiology】

- Exploration of lipidic bioactive substances
- Elucidation of phosphoinositide molecular species-specific signal transduction mechanism

【Developmental and Regenerative Biology】

- Discovery of the involvement of ceramide metabolism in mammalian early embryogenesis

【Molecular Cell Biology】

- *maea* affects head formation through ß-catenin degradation during early *Xenopus laevis* development.
- WNK1/HSN2 mediates neurite outgrowth and differentiation via a OSR1/GSK3β-LHX8 pathway.

【Stem Cell Regulation】

- Elucidation of the organization of self-advantageous niche by neural stem cells during development under hypoxia
- Discovery of a Sox17-downstream gene product Rasip1 which maintains hematopoietic stem cell-containing intra-aortic cell clusters in the midgestation

Department of Medical Chemistry

Professor	Katsumori Segawa, Ph.D.
Assistant Professor	Yugo Miyata, Ph.D.
JSPS Research Fellow	Sultan Cheryl Sophia, Ph.D.
Technical Assistant	Risa Kuribayashi, Moe Nishimoto
Secretary	Chikako Sawada

The asymmetrical distribution of phospholipids in plasma membranes is a fundamental architecture that confines phosphatidylserine (PtdSer) and phosphatidylethanolamine (PtdEtn) to the inner leaflet and enriches phosphatidylcholine (PtdCho) and sphingomyelin (SM) in the outer leaflet. There are three types of phospholipid translocases (flippase, floppase, and scramblase) that transfer phospholipids across the membrane lipid bilayer. Flippases translocate PtdSer and PtdEtn from the outer to the inner leaflet, whereas the translocation of PtdCho and SM from the inner to the outer leaflet has been proposed to be mediated by floppase. These molecules create the asymmetrical distribution of phospholipids in an ATP-dependent manner, while scramblases disrupt it by providing a path for the non-specific phospholipid transport between the two leaflets of the plasma membrane. We aim to identify various phospholipid translocases and elucidate their physiological and pathophysiological significances.

1. Background

Flippases establish and maintain asymmetrical distribution of PtdSer and PtdEtn by flipping them in one direction from the outer to the inner leaflet of the lipid bilayer. We found that ATP8A2, ATP11A, and ATP11C, which belong to the type IV P-type ATPase (P4-ATPase) family, are flippases that function at the plasma membrane in mammalian cells. These members form a stable flippase complex with CDC50A, an essential subunit for the most members of P4-ATPase family, and localize at the plasma membrane to flip the exposed PtdSer and PtdEtn. ATP8A2 is expressed explicitly in organs such as the brain and testis, while ATP11A and ATP11C are widely expressed throughout the body in mice and humans. Indeed, T-lymphoma cells doubly deficient in *ATP11A and ATP11C* lose plasma membrane flippase activity and fail to fip PtdSer on the cell surface after the transient PtdSer exposure. This indicates that plasma membrane flippases are essential for re-establishing the asymmetric distribution of PtdSer. Recently, Dr. Abe and his colleagues at Nagoya University determined the tertiary structure of ATP11C. This structure shows that PtdSer is captured in the lipid entry gate of ATP11C by the formation of salt bridges among several amino acids, including the amino acid (Q79) in the first transmembrane region and the head group of PtdSer, and that the subsequent conformational change of the flippase molecule upon hydrolysis of ATP enables PtdSer flipping to the inner leaflet. Last year, in collaboration with Dr. Shigeo Kure and his colleagues at the department of pediatrics, Tohoku University, we found a point mutation (Q84E) in flippase ATP11A in patients showing developmental delay and neurological deterioration. We reported that the mutation altered the substrate specificity of ATP11A flippase, and the mutant flipped PtdCho and totally changed the lipid organization in the outer leaflet of the plasma membrane.

2. Mechanisms for asymmetric distribution of phosphatidylserine in the plasma membranes by four flippases

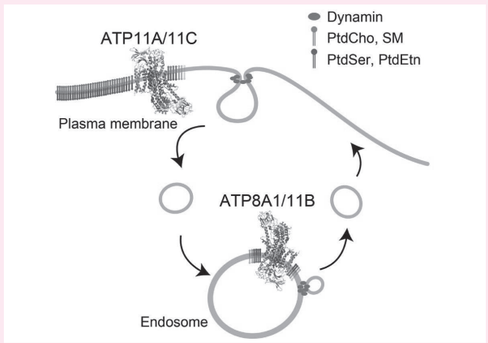
The P4-ATPases consist of 14-15 family members in human and mouse. Of these members, five, ATP8A1, ATP8A2, ATP11A, ATP11B, and ATP11C, have been detected to have flippase activity to translocate PtdSer and PtdEtn in both recombinant proteins and cell-based assays. ATP8A2, ATP11A, and ATP11C localize at the plasma membrane, while ATP8A1 and ATP11B localize mainly at the endosome and the Golgi apparatus. In addition, ATP8A2 is expressed explicitly in the brain and testis, whereas ATP8A1, ATP11A, ATP11B, and ATP11C are ubiquitously expressed throughout the body. These members require CDC50A to form functional flippase complexes, move to the plasma membrane and endosomes, and flip PtdSer and PtdEtn. Recently, we have reported that *ATP8A1-ATP11A-ATP11B-ATP11C* quadruple-deficient cells failed to internalize PtdSer into the inner leaflet of plasma membranes after the PtdSer exposure. When these quadruple-deficient cells expressed the plasma membrane flippases ATP11A or ATP11C, the exposed PtdSer was returned to the inner leaflet within 5 min at 37℃ and 15℃, and the asymmetric distribution was quickly re-established. In contrast, when endosome-localized ATP8A1 or ATP11B was expressed, the exposed PtdSer did not return to the inner layer at 15℃, and the asymmetric distribution took 30-60 minutes at 37℃ to be established. This re-establishment activity was inhibited by dynamin inhibitors, indicating that ATP8A1 and ATP11B remodel the PtdSer asymmetry via a membrane trafficking system such as endocytosis. Most types of cells have both endosome and plasma membrane flippases, suggesting that the asymmetric distribution of PtdSer is maintained by two independent systems at the plasma membrane (Highlight). Drs2p is a flippase in yeast that translocates PtdSer and localizes mainly to the endosome and the Golgi apparatus. In mammals, the ortholog of Drs2p is ATP8A1, but ATP11A and ATP11C have been further acquired during evolution. To ensure the complexed and higher-order functions of mammalian cells, it is assumed that ubiquitously expressed plasma membrane flippases, or activity to immediately re-establish the PtdSer asymmetry after its collapse, is required. Indeed, since deficiency or mutation of plasma membrane flippases causes severe and diverse diseases in mice and humans, the physiological importance of rapid re-establishment of the PtdSer asymmetry is crucial. Alternatively, ATP8A1 and ATP11B cannot compensate for the loss of plasma membrane flippase (the ability to establish an asymmetric distribution of PS within 5 min).

3. *Inefficient development of syncytiotrophoblasts in the Atp11a-deficient mouse placenta*

The P4-ATPases ATP11A and ATP11C function as flippases at the plasma membrane to translocate phosphatidylserine from the outer to the inner leaflet. We demonstrated that *Atp11a*-deficient mouse embryos died at approximately E14.5 with thin walled heart ventricles. However, the cardiomyocyte- or epiblast-specific Atp11a deletion did not affect mouse development or mortality. ATP11C may have compensated for the function of ATP11A in most of the cell types in the embryo. On the other hand, Atp11a, but not Atp11c, was expressed in the mouse placenta, and the Atp11a-null mutation caused poor development of the labyrinthine layer with an increased number of TUNEL-positive foci. Immunohistochemistry and electron microscopy revealed a disorganized labyrinthine layer with unfused trophoblasts in the Atp11a-null placenta. Human placenta-derived choriocarcinoma BeWo cells expressed the ATP11A and ATP11C genes. A lack of ATP11A and ATP11C eliminated the ability of BeWo cells to flip phosphatidylserine and fuse when treated with forskolin. These results indicate that flippases at the plasma membrane play an important role in the formation of syncytiotrophoblasts in placental development.

Highlight

ATP11A and ATP11C, the plasma membrane flippases, translocate PtdSer to the inner leaflet of within 5 min after PtdSer exposure. In contrast, expression of ATP8A1 and ATP11B, which localize at endosomes, re-establishes an asymmetric distribution of plasma membrane PtdSer over 30-60 minutes. This re-construction of the PtdSer asymmetry in plasma membranes by endosomal flippases is mediated by dynamin-dependent membrane trafficking. The asymmetry of plasma membrane PtdSer is maintained by two independent pathways mediated by membrane and endosomal flippases.



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3. Ochiai Y, Suzuki C, Segawa K, Uchiyama Y, Nagata S. Inefficient development of syncytiotrophoblasts in the Atp11a-deficient mouse placenta. Academy of Sciences of the United States of America. 2022.05; 119(18); e2200582119
4. Ryoden Y, Segawa K, Nagata S. Requirement of Xk and Vps13a for the P2X7-mediated phospholipid scrambling and cell lysis in mouse T cells. Proceedings of The National Academy of Sciences of The United States of America. 2022.02; 119(7); e2119286119. doi: 10.1073/pnas.2119286119.

Department of Biochemical Pathophysiology

Professor Takehiko Sasaki
Associate Professor Junko Sasaki
Assistant Professor Junya Hasegawa
Postdoctoral researcher Toshiyoshi Yamamoto, Emi Tokuda, Shin Morioka, Shogo Yanai

Lipids are responsible for cell compartmentalization through membrane formation, energy storage, and signal transduction within and between cells. Our laboratory is particularly interested in a group of phospholipids called phosphoinositides (Fig. 1). We have generated genetically modified mice with about 40 phosphoinositide kinases and phosphatases and are using them to study the pathogenesis of intractable diseases, including cancer, inflammatory diseases, and neurodegenerative diseases. In addition, we have developed a new mass spectrometry technique for phosphoinositides and are applying it to disease model mice and human disease samples to understand the mechanisms of pathogenesis caused by genetic abnormalities and environmental factors at the phospholipid molecular level (Fig. 2). Using these methods, we aim to deepen our understanding of the mechanisms of biological regulation by phospholipids and to develop therapeutic targets for intractable diseases, predictive markers for drug sensitivity, and markers for disease stratification (Fig. 3). In parallel with the phosphoinositide research, we are searching for phospholipids with novel structures. The bioactivity of some phospholipids discovered, the identification of synthesizing and degrading enzymes, and the identification of target proteins are in progress.

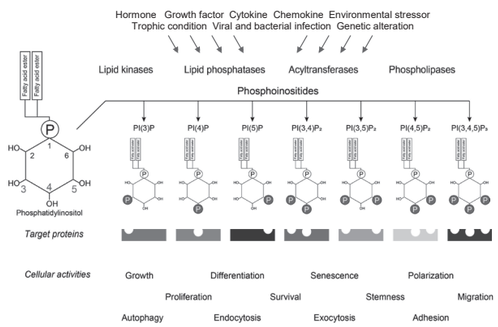

Fig.1 Phosphoinositide

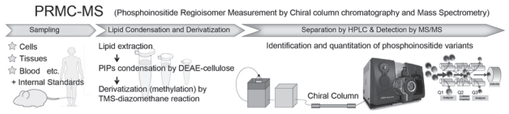

Fig.2 A new method for comprehensive analysis of phosphoinositide

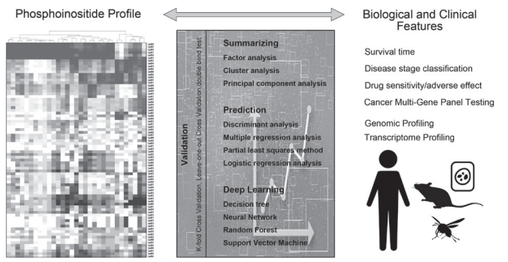

Fig.3 Phosphoinositide variants as molecular signatures for various diseases

Research Projects

1. Comprehensive measurement of phosphoinositide variants

We have devised a new method for analyzing phosphoinositide metabolism called PRMC-MS (Phosphoinositide Regioisomer Measurement by Chiral column chromatography and Mass Spectrometry). Previous methods of measuring and profiling phosphoinositides have produced results that cannot be easily applied to clinical or pathological samples from experimental animals. Even newer methods involving the use of mass-spectrometry which have made advances in some areas still reflect the problem of how to simultaneously quantify the acyl variants of individual regioisomers in biological samples.

The PRMC-MS method now solves this problem and points the way to an understanding of how these lipids influence cell functions. Using PRMC-MS, it is now possible to simultaneously measure all eight classes of phosphoinositides in a single sample. The highly sensitive nature of PRMC-MS allows for the detection of tiny but important changes in intracellular phosphoinositide levels, yielding data that shows that it can be applied to blood samples to track phosphoinositide signatures potentially related to inflammatory disease states.

PRMC-MS enables the comprehensive analysis of phosphoinositide acyl variants in various types of biological samples, including cultured cells surgical specimens, which can be used to throw a light on previously unrecognized disturbances of phosphoinositide fatty acyl profiles in cancerous tissue and to monitor their extracellular mobilization. Further study of the differing acyl variants and their conferring of protein binding properties could possibly also reveal how they activate a signaling pathway that favors cancer cell growth and survival and emerge as a target for cancer therapy. Thus, PRMC-MS may well illuminate the role played by phosphoinositides in the pathogenesis of cancers and inflammatory diseases. In addition, the use of PRMC-MS in the evaluation of phosphoinositide signatures at the acyl variant level in tissue and liquid biopsies may reveal biomarkers suitable for a wide variety of clinical applications.

In the future, applications such as the above may greatly facilitate drug development strategies based on the devising of a therapeutic agent that pinpoints a specific pathogenic phosphoinositide acyl variant, and thus open the way for much more accurate therapeutic methods and cures for patients suffering from a range of diseases that have proven difficult in the past.

2. Phosphoinositide metabolism to maintain femaleness

Mammalian sex is determined by the Y-linked Sry (Sex-determining region Y) gene. The supporting cell precursors of the fetal gonad differentiate into testicular Sertoli cells in the presence of Sry, while the cells differentiate into ovarian granulosa cells in the absence of Sry. These supporting cells commit to sexual differentiation of somatic and germ cells. Recent studies in mice have provided evidence that granulosa vs Sertoli cell fate decision is not necessarily permanent in postnatal life. In the adult ovary, Foxl2 (forkhead box L2) and estrogen receptors are required for maintaining granulosa cell fate by repressing male promoting signals, while in the adult testis, Dmrt1 (doublesex and mab-3 related transcription factor 1) and Sox9 (SRY-box 9) are required for maintaining Sertoli cell fate by repressing female promoting signals. Abolishing female promoting signals leads to loss of granulosa cell fate markers and up-regulation of Sertoli fate markers such as Sox9 and Dmrt1. We found that conditional knockout mouse mutants lacking phosphatidylinositol 3,4,5-trisphosphate (PIP_3) phosphatases exhibited female infertility. Histological examination of the mutant ovaries revealed that Sertoli-like cells emerged in the follicles. The female mutant mice had significantly higher levels of testosterone in the serum. Our results demonstrate that PIP_3 metabolism plays a key role in cell fate determination towards granulosa cells in the ovaries, and propose a possible etiology of DSD, disorders of sex development.

Publications

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Department of Developmental and Regenerative Biology

Professor	Hiroshi Nishina, Ph.D.
Lecturer	Satoshi Kofuji, Ph. D.
Assistant Professor	Yoshimi Okamoto, Ph. D.
Project Assistant Professor	Taku Tanaka, Ph. D.
	Jing Pu, Ph. D.
	Keiko Kanayama, Ph. D.

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

1. Research on early embryogenesis

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

2. Research on organogenesis

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

3. Research on organ homeostasis

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

Highlight

Roles of Hippo-YAP/TAZ in liver homeostasis and pathogenesis

The liver is an essential organ that plays a central role in metabolism and detoxification. It has an amazing regenerative capacity to withstand a variety of injuries. For example, it has the ability to eliminate senescent, transformed and damaged cells that cause cirrhosis and liver cancer. In addition, the liver maintains a constant size via regulation of cell number (Fig. 1). The discovery of the Hippo-YAP/TAZ signaling pathway is beginning to elucidate some of the molecular mechanisms underlying these phenomena, which have long remained unknown. About 20 years have passed since the discovery of this signaling pathway. In this review, we summarize the physiological and pathological roles of this signaling pathway from the viewpoint of maintaining liver homeostasis.

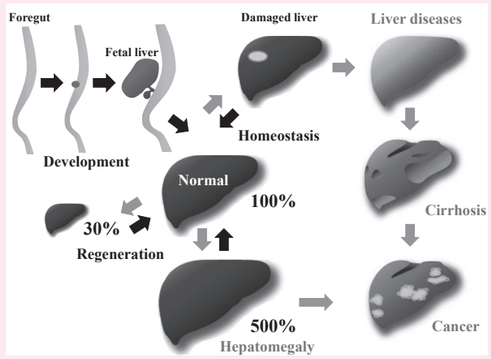

Fig.1.Conceptual diagram of liver development, regeneration and pathology

Publications

1. Hirotoshi Soyama, Miki Nishio, Junji Otani, Toshiko Sakuma, Shintaro Takao, Shigeo Hara, Takaaki Masuda, Koshi Mimori, Shinya Toyokuni, John P Lydon, Kazuwa Nakao, Hiroshi Nishina, Takumi Fukumoto, Tomohiko Maehama and Akira Suzuki (2022) Hippo-TAZ signaling is the master regulator of the onset of triple negative basal-like breast cancers. ***Proc Natl Acad Sci USA*** 119, e2123134119.

2. Caleb Kwame Sinclear, Junichi Maruyama, Shunta Nagashima, Kyoko Arimoto-Matsuzaki, Joshua Agbemefa Kuleape, Hiroaki Iwasa, Hiroshi Nishina, Hata, Yutaka (2022) Protein kinase C α activation switches YAP1 from TEAD-mediated signaling to p73-mediated signaling. ***Cancer Science*** 10.1111/cas.15285

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4. Chiyumi Oda, Kenya Kamimura, Osamu Shibata, Shinichi Morita, Yuto Tanaka, Toru Setsu, Hiroyuki Abe, Takeshi Yokoo, Akira Sakamaki, Hiroteru Kamimura, Satoshi Kofuji, Toshifumi Wakai, Hiroshi Nishina, Shuji Terai (2022) HBx and YAP Expression Could Promote Tumor Development and Progression in HBV-related Hepatocellular Carcinoma. ***Biochem. Biophys. Rep***. 32, 101352.

5. Sakurako Kobayashi, Nobuhiko Ogasawara, Satoshi Watanabe, Yosuke Yoneyama, Sakura Kirino, Yui Hiraguri, Masami Inoue, Sayaka Nagata, Yoshimi Okamoto-Uchida, Satoshi Kofuji, Hiromichi Shimizu, Go Ito, Tomohiro Mizutani, Shinichi Yamauchi, Yusuke Kinugasa, Yoshihito Kano, Yasuhiro Nemoto, Mamoru Watanabe, Kiichiro Tsuchiya, Hiroshi Nishina, Ryuichi Okamoto, Shiro Yui (2022) Collagen Type I mediated mechnotransduction controls epithelial cell fate conversion during intestinal inflammation. ***Inflammation and Regeneration*** 42, 49.

6. Hiroshi Nishina (2022) [review] Physiological and pathological roles of the Hippo-YAP/TAZ signaling pathway in liver formation, homeostasis and tumorigenesis. ***Cancer Science*** DOI: 10.1111/cas.15352

Department of Molecular Cell Biology

Professor Hiroshi Shibuya
Associate Professor Toshiyasu Goto
Assistant Professor Masahiro Shimizu

Overview

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

Introduction

Canonical Wnt signaling is very important in early *Xenopus* development. During early development before the gastrula stage, high Wnt activity at the dorsal side determines the organizer region. High Wnt activity increases expression of Spemann organizer genes, such as *nodal3.1* and *sia1*, and induces a secondary axis on the ventral side. Conversely, during late development after the gastrula stage, Wnt signaling inhibition at the organizer region by Wnt inhibitors, such as *cer1*, *frzb*, and *dkk1*, is required for head induction.

The key aspect of Wnt signaling is β-catenin protein stability. Dvl is recruited at the cell membrane and prevents β-catenin degradation under the Wnt-on state. Under the Wnt-off state, Axin1, Apc, Gsk3-β and Csnk1α1 form the destruction complex to phosphorylate β-catenin protein. Phosphorylated β-catenin is ubiquitinated by E3 ubiquitin ligases, such as Btrc, and is then degraded by the proteasome system.

The Glucose-Induced degradation Deficient (GID) complex also contributes to β-catenin ubiquitination and destruction. Wdr26, a scaffold protein in the GID complex, degrades β-catenin by binding to Axin1 and is required for head formation in *Xenopus*. There are two E3 ligases in the GID complex: Maea and Rmnd5a. Both human Maea and Rmnd5a ubiquitinate human β-catenin, and their knockdown increases human β-catenin stability in HEK 293T cells. Moreover, Rmnd5a knockdown in *Xenopus* reduced expression levels of genes that are necessary for forebrain development. Therefore, *Xenopus* Maea could also play an important role in β-catenin degradation in *Xenopus*. However, whether β-catenin protein degradation by Maea occurs in and affects *Xenopus* development remains unknown. Here, we investigated the effects of *Xenopus* Maea on early *Xenopus* development through β-catenin degradation.

1. Maea.S degraded and ubiquitinated β-catenin protein.

Xenopus laevis is an allotetraploid frog, and their two subgenomes, L (long) and S (short), were identified as sets of homeologous chromosomes with different lengths. The temporal expression patterns of *maea.S* and *maea.L* transcripts are almost similar during early development, although the *maea.S* transcripts are expressed more than those of *maea.L*. Therefore, we cloned *maea.S* and investigated its function in all experiments

In *Xenopus* embryos, *maea.S* reduced the amount of β-catenin protein. Furthermore, the immunoprecipitation assay revealed that Maea.S also bound to and ubiquitinated β-catenin. Moreover, RT-PCR analysis revealed that *maea.S* mRNA overexpression did not reduce β-catenin transcripts. These suggest that the decrease in β-catenin protein by Maea.S does not occur at the transcriptional level.

2. Overexpression of maea.S mRNA inhibited the effects of β-catenin

To confirm the effect of β-catenin protein degradation by Maea.S in *Xenopus* development, we investigated the effects of *maea.S* mRNA overexpression. Dorsally, *maea.S* mRNA overexpression decreased Wnt target gene expression at the gastrula stage (st. 10). However, the phenotypes of dorsal *maea.S* mRNA-injected embryos were similar to those of un-injected control embryos. These findings demonstrate that *maea.S* mRNA overexpression is sufficient to reduce the expression of Wnt target genes, but insufficient to change phenotypes. When we overexpressed *maea.S* mRNA into animal dorsal blastomeres of 8-cell embryos, the head structures, including the cement glands, of the injected embryos were enlarged (Figure 1). When we injected with a low dose of *β-catenin* mRNA into ventral blastomeres of 4-cell embryos, the injected embryos showed a secondary axis formation with complete or partial head structures. Co-injection with *maea.S* mRNA reduced the appearance rates of embryos that had the secondary axis with complete and partial head structures. Wnt target gene expression in embryos ventrally injected with *β-catenin* mRNA also decreased by co-injection with *maea.S* mRNA. These results suggest that *maea.S* may function as a gene that suppresses excessive Wnt activities through the degradation of β-catenin protein during early development.

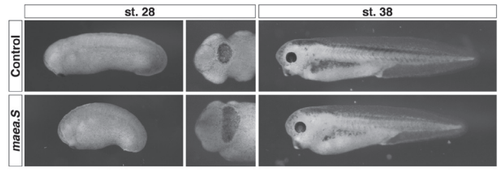

Figure 1. Overexpression of maea.S enlarged the head structures.

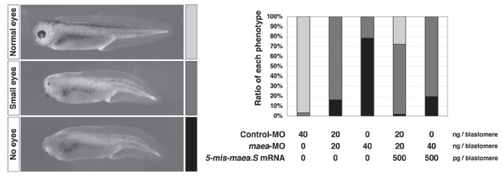

Figure 2. Knockdown of Maea.S inhibited the head formation.

3. Maea knockdown interfered with head formation

To investigate the effects of Maea knockdown on early development, we conducted experiments using *maea*-MO (morpholino oligonucleotides). Injection of *maea*-MO into animal dorsal blastomeres of 8-cell embryos interfered with head formation in a dose-dependent manner, and the head region phenotypes of the injected embryos were categorized as normal, mild (small eyes), or severe (no eyes) (Figures 2). Anterior development was rescued by co-injection with maea-MO and a MO-resistant form of *maea* mRNA, *5-mis-maea.S* mRNA, which contains five mismatch nucleotides in the MO binding site. These findings reveal that maea might contribute to head formation by inhibiting the Wnt activity through β-catenin protein degradation during early embryogenesis.

4. Maea.S might ubiquitinate unknown lysine residues of β-catenin

In vertebrates, the amino acid sequence of β-catenin involves 26 lysine residues, which are conserved among species. There are four lysine residues known as ubiquitinated sites in β-catenin protein. Both lysine residues 19 and 49 are ubiquitinated by Btrc and Jade1. Additionally, Siah1 ubiquitinates ß-catenin at lysine residues 666 and 671. Huwe1 and Shprh are also related to β-catenin protein degradation, but the sites they ubiquitinate have not been identified. To investigate whether Maea.S ubiquitinates novel lysine residues of β-catenin protein, we used a *β-catenin-4KRs* construct with four lysine to arginine mutations at 19, 49, 666, and 671 lysine residues. In results, *maea.S* reduced β-catenin-4KRs protein amounts. Moreover, Maea.S also bound to and ubiquitinated β-catenin-4KRs protein. Overexpression of *maea.S* reduced induction of both secondary axis and Wnt target gene expression by *β-catenin-4KRs* mRNA. These results demonstrate that β-catenin protein degradation by Maea.S might be due to β-catenin protein ubiquitination at unknown lysine residues.

Publications

Goto T. and Shibuya H. (2023). *maea* affects head formation through ß-catenin degradation during early *Xenopus laevis* development. **Dev. Growth Differ.** 65, 29-36.

Shimizu, M. and Shibuya, H. (2022). WNK1/HSN2 mediates neurite outgrowth and differentiation via a OSR1/GSK3β-LHX8 pathway. **Sci. Rep.** 12, 15858.

Goto T., Michiue T., and Shibuya H. (2022). *ccr7* affects both morphogenesis and differentiation during early *Xenopus* embryogenesis. **Dev. Growth Differ.** 64, 254-260.

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Department of Stem Cell Regulation

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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 2022 are mainly on three subjects: 1. Characterization of neural stem cells, 2. Characterization of fetal hematopoietic progenitors, and 3. Characterization of cancer stem cells and their niche.

Research Projects

1. Organization of self-advantageous niche by neural stem cells during development under hypoxia

Neural stem cells (NSCs) produce neurons and glial cells (astrocytes and oligodendrocytes), which are major cells in the brain. NSCs are maintained and expanded by self-renewal in order to avoid depletion during neurogenesis and astrogliogenesis. During midgestation, NSCs reside under hypoxia partly due to immature vasculature. The mechanisms by which NSCs efficiently self-renew under such circumstances have not fully been discovered. To tackle these problems, we first isolated NSCs from midgestation mouse brains and cultured them under the hypoxic condition. Neurosphere formation under the hypoxic condition was dramatically increased compared with that under the normoxic condition. Since neurosphere formation is a good indicator of the presence of NSCs, this result suggests a mechanism to maintain NSCs under hypoxia. Our further study surprisingly showed that NSCs cultured under hypoxia secreted vascular endothelial growth factor A (VEGF-A) and its amount was sufficient to induce NSC self-renewal. The experiments such as those using a VEGF-A inhibitor suggest that VEGF-A secreted from NSCs under the hypoxia promotes NSC self-renewal and contributes to maintenance and expansion of NSCs. Our results suggest that NSCs have adaptive potential to respond to hypoxia to organize self-advantageous niche involving VEGF-A when the vascular system is immature (Figure 1). This study will help to elucidate mechanisms underlying not only NSC maintenance but also vascular formation in the brain at the embryonic stages since VEGF-A is an important factor for vasculature development.

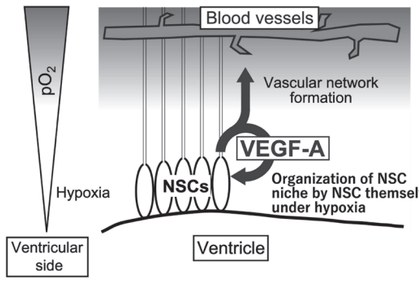

Figure 1. Organization of self-advantageous niche by NSCs under hypoxia

2. Sox17-downstream gene product Rasip1 maintains the hematopoietic stem cell-containing intra-aortic hematopoietic cell clusters in the midgestation mouse embryo

During mouse embryonic development, definitive hematopoiesis is first detected around embryonic day (E) 10.5 in the aorta-gonad-mesonephros (AGM) region, where hematopoietic stem cell (HSC)-containing clusters of cells emerge. We have previously shown that forced expression of the transcription factor Sox17, which is observed in such cluster-forming cells, promotes in vitro formation and maintenance of the clusters of cells with high hematopoietic potential. Sox17-downstream molecules, Notch1 and cell adhesion proteins, are found to be important for the maintenance of the undifferentiated state and the cluster formation. However, the role of Sox17 in hematopoiesis has not fully been explained. In 2022, we have focused on the *Rasip1* gene which was found, by RNA-sequencing, to be highly expressed in $CD45^{low}$c-Kit^{high} cells of E10.5 Sox17-expressing AGM cells. Its gene product Rasip1 is a protein that controls GTPase signaling, cell structure, and adhesion particularly in the vascular system. In the AGM region, as revealed by whole-mount immunostaining, Sox17 was expressed in part within the cluster-forming cells, whereas c-Kit and Rasip1 were expressed at the same time around the membrane of all cells in the clusters. The luciferase activity was diminished when the Sox17-binding sequence in the putative *Rasip1* enhancer region was mutated. Overexpression of Rasip1 in the cluster of cells gave them high hematopoietic potential, while knockdown of Rasip1 in Sox17-transduced cells impeded the cluster formation and diminished the hematopoietic ability. These results suggest that Rasip1 is functioning downstream of Sox17 and is involved in the maintenance of the hematopoietic activity of these cells. (Figure 2).

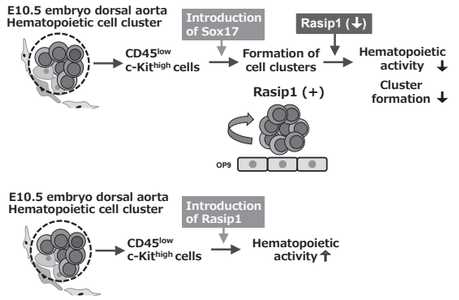

Figure 2. Model of the role of Sox17-induced Rasip1 expression in the hematopoietic ability of hematopoietic cell clusters

3. Identification of $CD45^{nega}$ $CD11b^{high}$ cells as a candidate of niche components self-organized by glioma stem cells

Cancer stem cells (CSCs) are a subset of tumor cells that are resistant to current chemo/radiotherapy and are capable of reconstructing original tumors. It has therefore been proposed that CSCs are a key driver of tumor recurrence and are understood as a promising target to eradicate cancers (Figure 3, Upper panel). Previously, we have reported that glioma CSCs (GSCs) secrete cytokines that induce monocytes and macrophages (Mϕ), by which and some other self-beneficial means GSCs establish the specialized microenvironments (i.e., GSC niche) for their self-expansion and glioma recurrence (Figure 3, Lower panel). Furthermore, our experiments using brain tumor-bearing mice have demonstrated that GSCs systemically stimulate erythropoiesis in the host bone marrow and spleen, then tumor-infiltrating Mϕs phagocytose the hemorrhaged erythrocytes, and thereby tumors achieve the sustainable replenishment of iron and oxygen which are essential niche factors for GSC maintenance. This result suggests that glioma is a systemic disease, and characterization of peripheral circulating immune cells recruited by GSCs could provide new insights into the mechanisms of glioma recurrence and the development of diagnostic/therapeutic strategies. This year, an immunocompetent mouse model was developed by intracranially transplanting mouse glioma cells which were originally established by transducing the oncogenic Ras^{V12} gene into $p53^{-/-}$ mouse brain cerebrum-derived astrocytes. When the profiles of immune cells in peripheral blood were analyzed, significantly lower levels of $CD45^{high}$ $CD11b^{low}$ monocyte-like cell fraction and significantly higher percentages of $CD45^{nega}$ $CD11b^{high}$ cell fraction were observed in glioma-bearing mice, and the latter fraction has been considered as bone marrow-derived microglial progenitor cells. Although the contribution of this new cellular fraction as a GSC niche is not well-elucidated yet, further investigation of this cell fraction could help us develop innovative diagnostics and therapeutics against glioma recurrence.

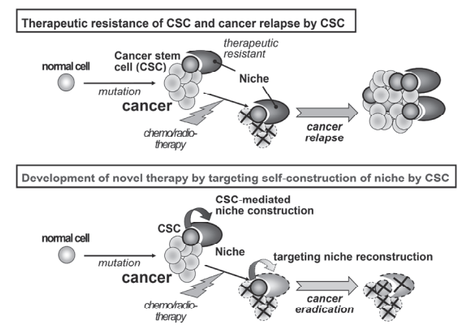

Figure 3. The concept of CSC-mediated niche construction and cancer eradication

Publications

[Original Article]

1. Aimaitijiang A, Tabu K, Wang W, Nobuhisa I, Taga T. Glioma cells remotely promote erythropoiesis as a self-expanding strategy of cancer stem cells. Genes Cells 27(1), 2022

[Review article]

1. Murota Y, Tabu K, Taga T. Cancer stem cell-associated Immune microenvironment in Recurrent Glioblastomas. Cells 11(13):2054, 2022
2. Wang W, Tabu K, Aimaitijiang A, Taga T. Therapy-resistant nature of cancer stem cells in view of iron metabolism. Inflamm Regen 42(1):34, 2022
3. Tabu K, Taga T. Cancer ego-system in glioma: an iron-replenishing niche network systemically self-organized by cancer stem cells. Inflamm Regen 42(1):54, 2022

Division of Advanced Pathophysiological Science

Aim and Scope

The intractable disease is a general term for diseases for which the etiology and pathogenesis are unknown and there are no effective preventive or therapeutic methods. The Division of Advanced Pathophysiological Science is working to deepen our understanding of the basic mechanisms of life phenomena and to develop new diagnostic, therapeutic, and preventive methods by elucidating intractable diseases' etiology and pathogenesis. The Division currently consists of five research fields and contributes to TMDU Priority Research Areas of "Rare Disease" and "Oral Science". Topics of research projects in each Department are as follows:

【Department of Biomolecular Pathogenesis】

- Using Fluoppi system, we revealed that Parkin (the causative gene product of hereditary Parkinson's disease) functions with UBE2D, UBE2L3, UBE2E1, UBE2E3 and UBE2C as partner E2s during ubiquitylation of damaged mitochondria.
- We found that BCAS3, the causative gene product of the hereditary neurodevelopmental disease HEMARS, is an autophagy-relevant factor that localizes on the isolation membrane, and that disease-related mutations in HEMARS inhibit BCAS3 localization on the isolation membrane.

【Biodefense Research】

- We found that new regulatory B cells emerge during systemic infection and that IL-10 produced by these cells enhances the innate immune cell supply in the early stages of infection.
- Using a human squamous cell carcinoma (mainly tongue and esophageal cancer) organoid library established in our laboratory, we have successfully selected chemotherapeutic drug-resistant & sensitive organoids.

【Neuropathology】

- We found that our original molecule PQBP5 is a relatively stable intrinsically disordered protein among nucleolar proteins and therefore essential for structural formation of the nucleolus.
- Using model mice for polyglutamine diseases such as Huntington's disease and spinocerebellar ataxia, we reveal that disease causative proteins bind to PQBP5 and cause nucleolar abnormality.

【Molecular Neuroscience】

- Synaptic pruning through glial synapse engulfment upon motor learning
- Generation of vasoactive intestinal peptide (VIP)^{tTA} knock-in mice

【Pathological Cell Biology】

- We developed a novel method for visualizing the autophagy maturation process.
- We have identified a compound that can induce GOMED, a proteolytic mechanism. We also found that the compound can improve the condition of polyglutamine disease model mice.

Department of Biomolecular Pathogenesis

Professor Noriyuki Matsuda
Associate Professor Koji Yamano
Assistant Professor Fumika Komatsuya

Various organelles are present and function in the cell. Many organelles are damaged in the process of their functions and are accumulate as damaged ones in the cell. In order to maintain organelle function, damaged organelles must be removed by selective autophagy. On the other hand, “degradation of organelles that are essential for survival” is a dangerous process. Therefore, the damaged organelle to be degraded must be correctly recognized and degraded under stringent control. In recent years, our understanding of the execution factors and physiological significance of selective organelle degradation are progressed. Ubiquitin and autophagy adaptors are important for selective organelle degradation. In addition, selective organelle degradation is closely related to neurodegenerative diseases such as Parkinson’s disease. We have been studying the molecular mechanisms of mitophagy, and have revealed that PINK1 and Parkin, the causative gene products of hereditary recessive Parkinson’s disease, label damaged mitochondria with ubiquitin, and lead the damaged mitochondria to autophagic degradation. We are going to elucidate the molecular mechanisms and the pathophysiological significance of selective organelle degradation from now on.

1. Fluoppi System

First, I would like to explain the Fluoppi (FLUOrescent based technology detecting Protein-Protein Interactions) system. This system was developed by Dr. Miyawaki and colleagues in RIKEN (Watanabe et al., Sci Rep 2017). In this experimental system, protein X fused with Azami Green (AG) and protein Y fused with Ash-tag are co-expressed in cells; when X and Y bind, liquid droplets are formed via multivalent interactions among Ash-X and AG-Y, and can be observed as intracellular fluorescence foci called Fluoppi foci (Figure 1A). The advantage of this experimental system is that the interaction between protein X and protein Y can be visualized and analyzed even in the living cells.

2. Molecular mechanisms of mitophagy was elucidated using the Fluoppi system

Molecular mechanisms that link ubiquitin and autophagic adaptors are important to provide selectivity for mammalian mitophagy. Serine/threonine kinase PINK1 (the causative gene product of hereditary Parkinson’s disease PARK6) and ubiquitin-ligase Parkin (the causative gene product of hereditary Parkinson’s disease PARK2) cooperate to ubiquitylate outer mitochondrial membrane proteins of damaged mitochondria, and they finally induce mitophagy. In mammalian cells, five autophagy adaptors (i.e., p62, NBR1, NDP52, OPTN/optineurin, and TAX1BP1) localize to Parkin-mediated ubiquitin-modified damaged mitochondria. All of them bind both ubiquitin and LC3, a protein covalently bound to phospholipids (phosphatidylethanolamine) to localize on autophagosomes. LC3 is an essential factor for autophagy formation and is conserved across species. The five autophagy adaptors described above do not localize to mitochondria under normal conditions. However, when mitochondria are damaged and ubiquitylated, the five autophagy adaptors are recruited onto mitochondria and interact with LC3 protein. Interestingly, their importance in mitophagy varies, and only NDP52 and OPTN are essential for Parkin-dependent mitophagy (Lazarou et al., Nature 2015). Thus, NDP52 and OPTN should have specific functions in directing damaged mitochondria to ubiquitin- and autophagy-dependent degradation. We tried to isolate autophagy factors that cooperated with OPTN using the Fluoppi system.

Ash-ubiquitin and AG-OPTN were co-expressed in cells to form liquid droplets. We then examined whether autophagy-related proteins were included in the ubiquitin- and OPTN-derived droplets. We found that the droplets contained ATG9, an essential factor for autophagosome formation (Yamano et al., JCB 2020). We next made OPTN mutant OPTN(ΔLZ) that can bind to ubiquitin and LC3, but cannot interact with ATG9. Cells in which all autophagy adaptors were disrupted (penta KO cells) were complemented with wild-type OPTN or mutant OPTN(ΔLZ), Parkin-mediated mitophagy was induced, and then the mitophagy activity was quantitatively measured. Mitophagy activity of OPTN(ΔLZ)-expressing cells was reduced to 1/4 of that in wild type OPTN-expressing cells. These results indicate that the OPTN - ATG9 interaction is important for Parkin-induced mitophagy.

3. Identification of E2s that cooperate with Parkin in mitophagy

Parkin is a ubiquitin-ligating enzyme (E3). All E3s function in concert with a ubiquitin-activating enzyme (E1) and a ubiquitin-conjugating enzyme (E2). There are thirty different types of E2s in mammalian cells. Although several E2s have already been reported to cooperate with Parkin during mitophagy, the E2s identified as Parkin partner were different among the papers. Therefore, we tried to use the Fluoppi system to determine the E2s that function as genuine partner of Parkin in mitophagy. In general, physical binding between E2 and E3 is transient. To stabilize the binding between E2 and E3, we introduced a mutation (C431S) in the catalytic center of Parkin (Cys431) and prepared Parkin (C431S) mutant fused with Ash-tag (hereafter called Ash-Parkin). We also fused twenty-eight different types of E2 with Azami Green and named them AG-E2s. Ash-Parkin and AG-E2s were co-expressed in cells and observed whether Fluoppi foci (fluorescent droplets) were formed or not. Under normal (steady state) conditions, all E2s examined did not form Fluoppi foci with Parkin. In contrast, when mitochondrial membrane potential was disrupted to induce mitophagy, several E2s and Parkin formed Fluoppi foci (Figure 1B). These results are reasonable, as structural analysis has showed previously that Parkin is kept in an inactive form which is unable to interact with E2 via autoinhibition. Fluopi analysis narrowed the list of candidates E2 down from thirty to eleven. Next, we tried to reconstitute Parkin-mediated ubiquitylation of damaged mitochondria in the cell-free experiment. Namely, mitochondria prepared from cells were reacted with purified ubiquitin, E1, eleven types of E2, and Parkin. Seven of the eleven E2s isolated by the Fluoppi assay could cooperate with Parkin to ubiquitylate mitochondrial outer membrane proteins. Importantly, normal (healthy) mitochondria were not ubiquitinated in this cell free analysis, whereas the damaged mitochondria were ubiquitylated. These results indicate that the E2s acting as partner for Parkin during mitophagy are UBE2Ds, UBE2L3, UBE2E1, UBE2E3, and UBE2C (Hayashida et al., JBC 2023).

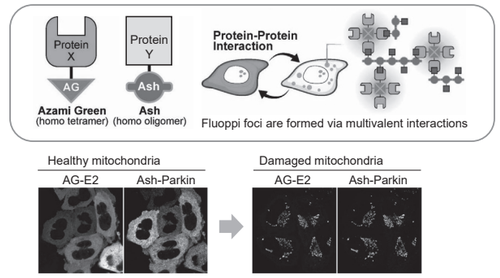

Figure 1: Interaction between Parkin and several E2s depends on mitochondrial damage.
(A) schematic diagram of the Fluoppi system. Protein X fused to Azami Green (AG) and a Protein Y fused to Ash-tag are co-expressed in cells. If X and Y bind together, fluorescent droplets are formed in the cell through multivalent interactions between AG-X and Ash-Y.
(B) We found an interaction between Parkin and UBE2L3 E2 upon mitochondrial damage. E2 (UBE2L3) fused with AG and Parkin fused with Ash-tag were coexpressed in cells and were examined whether Fluoppi foci were made. UBE2L3 and Parkin did not interact al all and thus no Fluoppi foci was observed under normal conditions, whereas when mitochondrial membrane potential decreases, they bind and form a bright fluorescent droplet (Fluoppi foci) in cells.

4. Other research proposal

In addition to the research topics described above, we have been studying several themes related to selective organelle degradation.

(1) We have isolated BCAS3 as a novel factor that migrates to the isolation-membrane near damaged mitochondria during mitophagy (Kojima et al., Autophagy 2021). We are currently studying molecular function of BCAS3 and its physiological significance.

(2) It is known that TBK1 kinase binds to OPTN (autophagy adaptor protein during mitophagy) and phosphorylates OPTN. We thus are studying the role of TBK1 in Parkin-mediated mitophagy.

(3) Ubiquitylation is important in peroxisome-specific autophagy (pexophagy) as well. We are studying the molecular mechanisms underlying pexophagy, especially how ubiquitin modification is converted into a signal for pexophagy progression.

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1. Elucidation of ubiquitin-conjugating enzymes that interact with RBR-type ubiquitin ligases using a liquid-liquid phase separation-based method. Hayashida R, Kikuchi R, Imai K, Kojima W, Yamada T, Iijima M, Sesaki H, Tanaka K, Matsuda N, Yamano K. J Biol Chem. 299(2):102822, 2023.

Department of Biodefense Research

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Assistant Professor	Masashi Kanayama, Ph.D.
Adjunct Lecturer	Nobuyuki Onai, Ph.D.
Adjunct Lecturer	Yasuhiro Murakawa, MD, Ph.D.
Research Technician	Kisho Shiseki
Research Technician	Toyoki Hayashi
Secretarial Assistant	Hisako Kamioka

Our research projects focus on understanding the dynamic maintenance and transfiguration of homeostasis in the living body. Our goal is to define the homeostasis mechanism under conditions of health and disease. To accomplish this goal, we are trying to clarify the molecular basis of induction and failure of homeostasis by focusing on immune cells in particular **myeloid cells** (dendritic cells and macrophages), **tissue stem cells** and **cancer stem cells** in blood and epithelium, 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 the prevention and treatment of disease.

1. Research on myeloid cells

1) Identification of novel sources of dendritic cells and macrophages

Dendritic cells (DCs) consist of conventional DCs (cDCs) and plasmacytoid DCs (pDCs), both of which play critical regulatory roles in the immune system. cDCs exhibit prominent antigen-presenting ability, whereas pDCs are characterized by their capacity to produce large amounts of type I interferons (IFNs) in response to viral and self-nucleic acids. We have discovered the DC progenitors in the mouse bone marrow, and named them common DC progenitors (CDPs) (***Immunity*** 2013; ***Nat Immunol*** 2007). Interestingly, we found that CDPs are divided into 2 subpopulations. One is M-CSF receptor (R)$^{+}$ CDPs mainly producing cDCs, and the other M-CSFR^{-}CDPs producing many pDCs.

During stress response, monocytes actively influx into various tissues and differentiate into macrophages, which are involved in inflammation, tissue repair, and cancer growth. In addition to CDPs, we recently found human common monocyte progenitors (cMoPs) in human bone marrow and umbilical cord blood (***Immunity*** 2017; ***Int Immunol*** 2018). Human cMoP gives rise to only monocytes but not other hematopoietic cells including DCs. Given that monocytes are involved in chronic myelomonocytic leukemia (CMML) and monocyte-derived tumor-associated macrophages (TAMs) promote tumor development, we, in collaboration with a pharmaceutical company, have generated an antibody-drug conjugate (ADC) that selectively targets human cMoP. When this ADC was administered to the CMML PDX model, leukemia cells almost completely disappeared from the bone marrow and peripheral blood. In addition, upon ADC administration into tumor-bearing humanized mice, both peripheral blood monocytes and intratumoral TAMs disappeared, leading to the shrinkage of tumor mass (***Front Immunol*** 2021). Since monocytes are also involved in various inflammatory diseases, the application of human monocyte lineage-specific ADCs to these diseases is also expected.

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

The decline in tissue regeneration and homeostasis associated with life-stage progression is closely related to the functional alteration of macrophages. Microglia, a macrophage in the brain, is actively contributing to the brain development and maintenance during young age (regenerative microglia). However, with age, microglial inflammatory trait becomes prominent with impaired phagocytosis and brain-derived neurotrophic factor (BDNF) production, etc (inflammatory microglia).

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

3) Elucidation of the mechanism of myeloid cell production during infection

In systemic infections, the production of myeloid cells is markedly increased to enhance innate immunity (Emergency Myelopoiesis, EM) to rapidly eliminate pathogens that have invaded the body. Our research group has found that interleukin 10 (IL-10) produced by regulatory B cells that emerge during infection (***J Exp Med*** in press) enhances the supply of myeloid system cells (**Figure 1**).

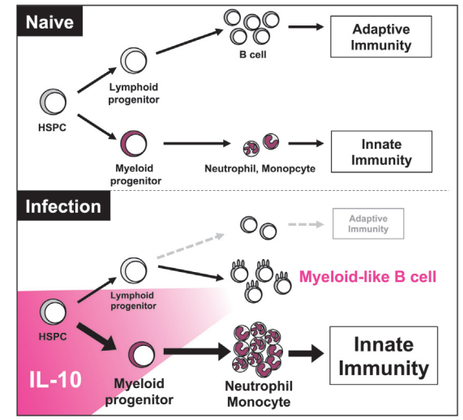

Fig.1 Increased production of innate immune cells by a new B cell subset that emerges during infection
Kanayama M et al., *J Exp Med* in press, cited from Graphic Abstract with modifications

2. Research on tissue stem cells

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

Even under the steady-state, type I interferons (IFNs) are consistently produced, albeit in trace amounts, so called "physiologic type I IFNs". We previously reported that the physiologic type I IFNs induce proliferation and exhaustion in hematopoietic stem cells (HSCs), and that interferon regulatory factor-2 (IRF2), a transcriptional suppressor of type I IFN signaling, preserves the self-renewal and multi-lineage differentiation capacity of HSCs (***Nat Med*** 2009). Based on this achievement, we examined the significance of physiologic type I IFNs in intestinal stem cells (ISCs) and found that it reduces the number and function of ISCs, resulting in the promoted differentiation into secretory progenitors (***Nat Cell Biol*** 2020). Similarly, physiologic type I IFNs impaired the stemness of colonic stem cells (CSCs), leading to defective colonic regeneration with lethality in a DSS colitis model (***Sci Rep*** 2020). Furthermore, we have found that chronic type I IFN signaling induces abnormalities such as alopecia and fibrosis in the skin, and we are currently investigating its relationship to human autoimmune and autoinflammatory diseases (unpublished).

2) Establishment of human tongue cancer organoid biobank

Squamous cell carcinoma occurs in the oral cavity, esophagus, lungs, and cervix. Tongue cancer accounts for about 60% of oral cancer, and the 5-year survival rate is extremely low for advanced tongue cancer, and the recurrence rate after radical treatment is also high. Similarly, squamous cell carcinoma of the esophagus, which is characteristic of Asian countries including Japan, has a very high recurrence rate after curative treatment. As a multicenter collaborative study, our research group has succeeded in constructing an organoid library specialized for human tongue cancer and human esophageal squamous cell carcinoma, which has never been reported (34 cases of tongue cancer and 24 cases of esophageal cancer, unpublished). In addition, we have established cancer organoids that are resistant to anticancer drugs used in clinical treatment (4 cases of tongue cancer and 4 cases of esophageal cancer, ongoing). Using these unique resources, the elucidation of the mechanism for acquiring anticancer drug resistance and the search for drug discovery is in progress.

Publications

1. Kanayama M, Izumi Y, Akiyama M, Hayashi T, Atarashi K, Roers A, and Ohteki T. Myeloid-like B cells boost emergency myelopoiesis through IL-10 production during infection. ***J Exp Med*** in press

2. Cui G, Shimba A, Jin J, Ogawa T, Muramoto Y, Miyachi H, Abe S, Asahi T, Tani-Ichi S, Dijkstra JM, Iwamoto Y, Kryukov K, Zhu Y, Takami D, Hara T, Kitano S, Xu Y, Morita H, Zhang M, Zreka L, Miyata K, Kanaya T, Okumura S, Ito T, Hatano E, Takahashi Y, Watarai H, Oike Y, Imanishi T, Ohno H, **Ohteki T**, Minato N, Kubo M, Holländer GA, Ueno H, Noda T, Shiroguchi K, Ikuta K. A circulating subset of iNKT cells mediates antitumor and antiviral immunity. **Sci Immunol** 2022, 7:eabj8760. doi: 10.1126/sciimmunol.abj8760.

Personnel Changes

Moving in:
Nakagawa S, Ph.D. student in the Department of Gastrointestinal Surgery (D2) (April 1, 2022).

Yamada Y, Short-term exchange student from Tokyo University of Pharmacy and Life Sciences (September 12, 2022).

Department of Neuropathology

Professor	Hitoshi Okazawa
Project Lecturer/Part-time Lecturer	Haruhisa Inoue, Masaki Sone, Kyota Fujita
Project Associated professor	Hidenori Homma
Lecturer	Hikari Tanaka

[Outline]

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

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

[This year's progress]

Mind your Qs: polyQ-binding protein 5 scaffolds the nucleolus

Researchers from Tokyo Medical and Dental University (TMDU) find that PQBP5/NOL10, an intrinsically disordered protein, scaffolds the nucleolus under normal and stressed conditions and is bound by polyglutamine disease proteins

Everyone has that one friend who's the life of the party, bringing people together and keeping everyone connected. Now, researchers from Japan find that an unusually structured protein plays a similar role in bringing a diverse group of proteins together and keeping them connected and functional.

In a study published recently in *Nature Communications*, we have revealed that an intrinsically disordered protein (IDP) is crucial for the stability of an organelle called the nucleolus.

The nucleolus is critical for transcribing ribosomal DNA, which encodes crucial components of the ribosome, an essential organelle for cellular maintenance, differentiation, and stress responses. Many proteins that are part of the nucleolus are IDPs that are susceptible to deformation and dysfunction in response to stressors such as temperature changes, low oxygen conditions, or dehydration.

We previously identified the IDP polyglutamine binding protein 5 (PQBP5), also known as nucleolar protein 10 (NOL10), in a screen for proteins that bind to polyglutamine (polyQ) tract sequences in proteins that cause

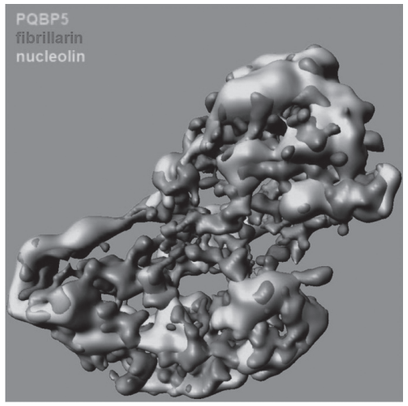

Figure 1 PQBP5 is an essential structural component of the nucleolus. This 3D image of a single nucleolus shows the interactions among three nucleolar proteins: PQBP5 (green) forms a lattice-like meshwork ball that anchors assembly of fibrillarin (red) and nucleolin (blue).

polyQ diseases. PQBP5/NOL10 was later found to be a component of the nucleolus. the integrity of which has been implicated in the pathophysiology of neurodegenerative diseases.

To determine whether PQBP5/NOL10 is involved in maintaining the structural integrity of the nucleolus, the researchers investigated its molecular characteristics, nucleolar sublocalization, relationship with other nucleolar proteins, and stress responses.

Unexpectedly, we found that PQBP5/NOL10 is a core structural element of the nucleolus, forming a meshwork that supports other nucleolar substructures. Even more intriguingly, unlike other nucleolar proteins that disperse to the nucleoplasm under osmotic stress conditions, PQBP5/NOL10 remains in the nucleolus and anchors reassembly of the nucleolar structure.

In addition, the researchers found that PQBP5/NOL10 can essentially be sponged up by polyQ disease proteins, both in cells and in mice. This leads to deformation or even disappearance of the nucleoli.

Taken together, these findings indicate that PQBP5/NOL10 is an essential protein needed to maintain the structure of the nucleolus.

Given that polyQ proteins form aggregates with a dense core that often excludes polyQ-binding proteins, PQBP5/NOL10 may initially interact with soluble forms of these proteins before being pulled into larger inclusions. Aggregation inhibitors that prevent inclusion formation could therefore affect PQBP5/NOL10 distribution, and thus nucleolar stability, providing a novel approach to treating polyQ diseases.

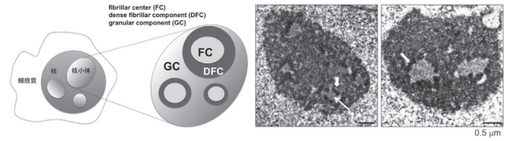

Fig.1 Structure of Nucleolus
The nucleolus has a layered structure called FC, DFC and GC.

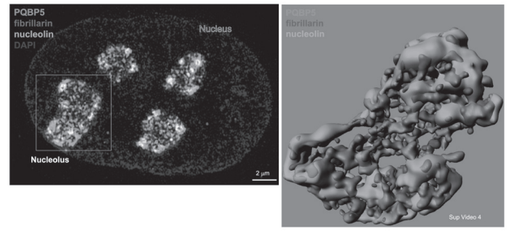

Fig.2 Distribution of PQBP5 inside the nucleolus
Left) Distribution of PQBP5, fibrillarin, and nucleolin by super-resolution microscopy.
Right) A three-dimensional image by super-resolution microscope data with the image analysis software Imaris.

Department of Molecular Neuroscience

Professor Kohichi Tanaka
Assistant Professor Yuichi Hiraoka
Assistant Professor Tetsuo Ohnishi

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 (Fig.1).

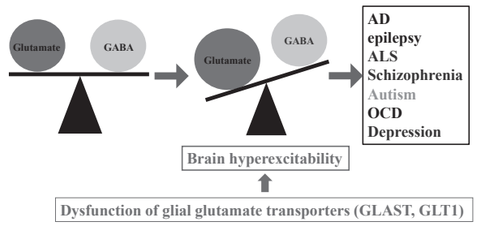

Fig.1 Glutamate transporter dysfunction leads to neuropsychiatric diseases

2. Role of glial engulfment in motor learning

Synaptic pruning is a fundamental process of neuronal circuit refinement in learning and memory. Accumulating evidence suggests that glia participates in sculpting the neuronal circuits through synapse engulfment. However, whether glial involvement in synaptic pruning has a role in memory formation remains elusive. In this study, we developed a genetic strategy for visualizing phagocytic events to reveal the involvement of glial phagocytosis in the pruning process. We found that Bergmann glia (BG) have a high phagocytic capacity in naive healthy mouse cerebellar cortex (Fig.2). Furthermore, we found that synaptic engulfment by cerebellar BG frequently occurred upon cerebellum-dependent motor learning in mice. We observed increases in pre- and postsynaptic nibbling by BG along with a reduction in spine volume after learning. Pharmacological blockade of phosphatidylserine (PS)-dependent phagocytosis with Annexin V inhibited both the spine volume reduction and improvement of the learned behavior. These results indicate that BG contribute to the refinement of the mature cerebellar cortical circuit through synaptic engulfment during motor learning.

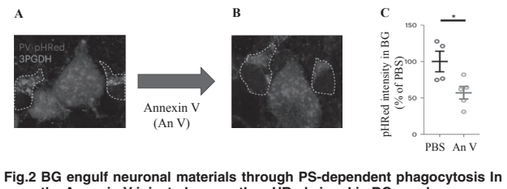

Fig.2 BG engulf neuronal materials through PS-dependent phagocytosis In the Annexin V-injected group, the pHRed signal in BG was lower.

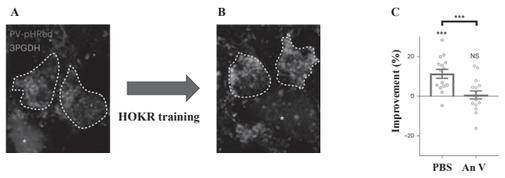

Fig.3 BG engulfment of neuronal materials increases after learning
A and B: pHRed intensity in BG increased after the horizontal optokinetic response (HOKR) training
C: The increase in HOKR gain was not observed in the Annexin V group.

3. Generation of vasoactive intestinal peptide (VIP)^{tTA} knock-in mice

The suprachiasmatic nucleus (SCN), the central circadian clock in mammals, is a neural network consisting of various types of GABAergic neurons, which can be differentiated by the co-expression of specific peptides such as vasoactive intestinal peptide (VIP), arginine vasopressin (AVP), and gastrin-releasing peptide (GRP). VIP is expressed in about 10% of SCN neurons and has been considered as a critical factor for the circadian rhythmicity and synchronization of individual SCN neurons. However, the precise mechanisms of how VIP neurons regulate SCN circuits remain incompletely understood. Here, we generated *Vip*^{tTA} knock-in mice that express tetracycline transactivator (tTA) specifically in VIP neurons by inserting tTA sequence at the start codon of *Vip* gene. The specific and efficient expression of tTA in VIP neurons was verified using EGFP reporter mice. In addition, combined with *Avp-Cre* mice, *Vip*^{tTA} mice enabled us to simultaneously apply different genetic manipulations to VIP and AVP neurons in the SCN. Furthermore, because the transcription of the endogenous *Vip* coding sequence was blocked in the *Vip*^{tTA} allele, VIP was completely absent in homozygous mice of the line. Consistently, homozygous *Vip*^{tTA} mice showed impaired circadian behavioral rhythms similar to those of *Vip* knockout mice, such as attenuated rhythmicity and shortened circadian period. In contrast, heterozygous mice demonstrated normal circadian behavioral rhythms comparable to wild-type mice. These data suggest that *Vip*^{tTA} mice are a valuable genetic tool to express exogenous genes specifically in VIP neurons in both normal and VIP-deficient mice, facilitating the study of VIP neuronal roles in the SCN neural network.

Publications

[Original papers]

1. Ito, T., Hiramatsu, Y., Aida, T., Kushima, I., Yoshida, M., Yoshimi, A., Tanaka, K., Ozaki, N., Noda, Y. Astrotactin2 (ASTN2) regulates emotional and cognitive functions by affecting neuronal morphogenesis and monoaminergic systems. *J Neurochem* (in press)
2. Morizawa, Y., Matsumoto, M., Nakashima, Y., Endo, N., Aida, T., Ishikane, H., Beppu, K., Moritoh, S., Inada, H., Osumi, N., Shigetomi, E., Koizumi, S., Yang, G., Hirai, H., Tanaka, K., Tanaka, K.F., Ohno, N., Fukazawa, Y., Matsui, K. Synaptic pruning through glial synapse engulfment during motor learning. *Nature Neurosci* 25. 1458-1469, 2022.
3. Peng, Y., Tsuno, Y., Matsui, A., Hiraoka, Y., Tanaka, K., Horike, S., Daikoku, T., Mieda, M. Cell type-specific genetic manipulation and impaired circadian rhythms in *Vip*^{tTA} knock-in mice. *Frontiers in Physiology* 13. 895633, 2022
4. Ishida, S., Zhao, D., Sawada, Y., Hiraoka, Y., Mashimo, T., Tanaka, K. Dorsal telencephalon-specific Nprl1- and Nprl3-knockout mice: novel mouse models for GATORopathy. *Hum Mol Genet* 31. 1519-1530, 2022.
5. Irie, M., Itoh, J., Matsuzawa, A., Ikawa, M., Kiyonari, H., Kihara, M., Suzuki, T., Hiraoka, Y., Ishino, F., Ishino, T.K., Retrovirus-derived RTL5 and RTL6 genes are novel constituents of the innate immune system in the eutherian brain. *Development* 149. dev200976, 2022
6. Tamura, A., Ito, G., Matsuda, H., Nibe-Shirakihara, Y., Hiraoka, Y., Kitagawa, S., Hiraguri, Y., Nagata, S., Aonuma, E., Otsubo, K., Nemoto, Y., Nagaishi, T., Watanabe, M., Okamoto, R., Oshima, S., Zranb1-mutant mice display abnormal colonic mucus production and exacerbation of DSS-induced colitis. Biochem *Biophys Res Commun*. 628. 147-154, 2022

Department of Pathological Cell Biology

Professor	Shigeomi SHIMIZU
Project Junior Associate Professor	Satoru TORII
Junior Associate Professor	Hirofumi YAMAGUCHI
Project Junior Associate Professor	Masatsune TSUJIOKA, Shinya HONDA
Assistant professor	Yoichi NIBE
Project Assistant Professor	Hajime SAKURAI, Min Kyong SHIN

The research in our laboratory is focused on four main areas: 1) elucidation of the physiological and pathological significance of Golgi-membrane-associated degradation (GOMED), a cellular function that we have discovered, 2) elucidation of the molecular mechanism of autophagy and its physiological and pathological significance, 3) analysis of cell death mechanisms and development of therapeutic agents for diseases derived from its failure, and 4) exploration of new functions of organelles represented by mitochondria and the Golgi apparatus.

〈**Research Projects**〉

1, Molecular Mechanism and Physiological Function of GOMED

GOMED is a proteolytic mechanism executed by structures similar to autophagy. However, it differs significantly from autophagy in terms of the induced stimuli, executing molecules, and types of degraded proteins. In addition, autophagy is executed using the endoplasmic reticulum membrane, whereas GOMED is executed using the Golgi membrane (Figure 1). The Golgi apparatus normally functions as an integrated cis-, medial-, and trans-membrane to make appropriate modifications to secretory and plasma membrane proteins and transport them to their proper locations. However, when secretory proteins or other proteins are transported in excess, or when proteins become abnormal, GOMED is activated and a portion of the trans membranes diverges from the medial membranes, spheroidizes, and envelops the abnormal protein. The wrapped proteins are subsequently degraded by lysosomal enzymes. Autophagy and GOMED coexist in a single cell and are used differently depending on the type of stimulus applied to the cell or the type of substrate to be degraded.

The molecular mechanism of GOMED is well conserved from yeast to mammalian cells, and molecules such as Ulk1 and Beclin 1 are important in the early stage. During GOMED execution, the signal is activated in the following order: dephosphorylation of serine at 637 of Ulk1, phosphorylation of serine at 746, and translocation to the Golgi membrane. In addition, the Wipi3 is activated downstream and is involved in the deformation of the Golgi membranes. The curved Golgi membrane envelopes cytoplasmic components and proteins in the Golgi, which are eventually degraded by lysosomal enzymes.

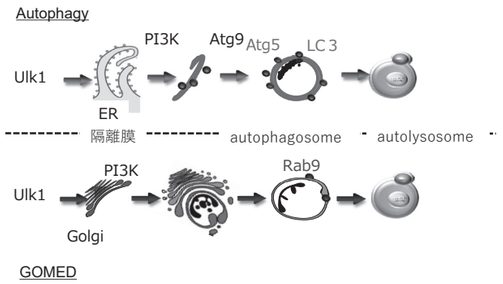

Figure 1. Hypothetical model of autophagy and GOMED.
Autophagy requires Atg5 and is originated from the ER membrane. In contrast, GOMED occurs independently of Atg5 and is originated from the Golgi membrane.

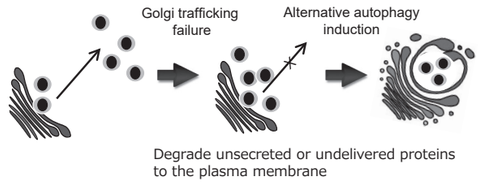

Figure 2. Physological role of GOMED

The most important difference between autophagy and GOMED is the difference in degradation substrates. Autophagy mainly degrades cytosolic proteins such as p62 and LC3. In contrast, in GOMED, the substrate molecules are those transported via the Golgi apparatus (Figure 2). A typical example of such GOMED function is the regulation of insulin secretion. Insulin is synthesized in insulin-secreting cells (β-cells) of the pancreas and secreted via the Golgi apparatus. When the glucose concentration around the cells decreases (i.e., blood glucose level falls), insulin secretion is suppressed to prevent further low glucose. At this time, GOMED is induced in the β-cell to alleviate insulin retention. GOMED is also involved in neuronal homeostasis. This finding is evident from the fact that in mice lacking the neuron-specific Wipi3 gene, Purkinje cells in the cerebellum degenerated and dropped out, resulting in the appearance of behavioral abnormalities. GOMED contributes to the constant degradation of ceruloplasmin, an iron transport protein, and failure of this degradation leads to the development of iron deposition neurodegenerative diseases.

2, Drug discovery based on GOMED

Since modulation of GOMED causes neurodegenerative diseases, we thought that it might be possible to treat neurodegenerative diseases by activating GOMED. In fact, when GOMED-inducing compounds were searched for and administered to model mice with polyglutamine disease, they were found to alleviate polyglutamine accumulation, improve neurodegeneration, and normalize behavioral abnormalities. We are currently investigating the application of GOMED to diseases other than neurodegenerative disorders.

3, Analysis of Cell Death

The major cell death that occurs in living organisms is apoptosis, but recent analyses are revealing the importance of non-apoptotic cell death. Our laboratory has discovered autophagic cell death and mitochondria-mediated necrosis, and has been analyzing these cell deaths. Recently, we discovered a new execution mechanism of cell death by loss of cell adhesion (anoikis).

4, Exploring New Functions of Organelles

Conventionally, organelles such as mitochondria and Golgi apparatuses have been thought to each have their own unique functions within the cell. However, the development of super-resolution microscopy has revealed that contact surfaces exist between organelles. We have found that crosstalk between the Golgi and mitochondria and between the Golgi and endoplasmic reticulum, and are analyzing the detailed mechanisms of mitochondrial and endoplasmic reticulum regulation by the Golgi.

Highlight

A Novel Method to Visualize the Maturation Processes of Autophagy and GOMED

When autophagy and GOMED degrade intracellular components, they sequester them from the cytoplasm by wrapping in a sequestration membrane. Until now, there has been no research method to properly assess the progress of this sequestration. Therefore, we constructed a measurement system in which fluorescent molecules are placed inside the sequestration membrane and the progress of sequestration is evaluated by the extent of the fluorescent molecules. Specifically, fluorescent proteins such as GFP are expressed in cells. Then, photobleaching (a technique in which fluorescent proteins are quenched by strong excitation) is applied to a portion of the cell. As a result, fluorescent proteins not wrapped in the sequestration membrane are dicreased by free diffusion, while fluorescent proteins wrapped in the sequestration membrane remain in the sequestration membrane and emit strong fluorescence. This technique is named FLAD (FLIP-based autophagy detection).

Publications

[Original paper]
1, Nickel particles are present in Crohn's disease tissue and exacerbate intestinal inflammation in IBD susceptible mice. Matsuda H, Nibe-Shirakihara Y, Tamura A, Aonuma E, Arakawa S, Otsubo K, Nemoto Y, Nagaishi T, Tsuchiya K, Shimizu S, Ma A, Watanabe M, Uo, M, Okamoto R. BBRC 592, 2022 74-80
2, Absence of ULK1 decreases AMPK activity in the kidney, leading to chronic kidney disease progression. Yanagi T, Kikuchi H, Susa K, Takahashi N, Bamba H, Suzuki T, Nakano Y, Fujiki T, Mori Y, Ando F, Mandai S, Mori T, Takeuchi K, Honda S, Torii S, Shimizu S, Rai T, Uchida S, Sohara E. Genes Cells 2022, 12989.
3, FLIP-based autophagy-detecting technique reveals closed autophagic compartments. Tajima-Sakurai H, Arakawa S, Noguchi S, and Shimizu S. Scientific Reports 2022, 12, 22452.

Division of Biological Data Science

Over the last decade, remarkable strides have been made for data science in the field of biology and medicine. The mission of our division is to elucidate the etiology of diseases and develop novel treatments through integrated analysis of biological data. We obtain omics data such as genome, transcriptome, and proteome by the state-of-art technologies such as single-cell analysis, molecular structure analysis, and bioimaging. In addition, using the advanced data analytics techniques such as AI, we will scientifically clarify what has been called "predisposition to disease" to realize personalized medicine and to develop disease prevention methods.

【New Departments】
Computational and Systems Biology
Advanced Nanomedical Engineering

【Existent Departments】
Structural Biology
Functional Genomics
Genomic Function and Diversity
Bio-informational Pharmacology

【Main Research Activities】
Press releases in 2022

- Development of a method to identify genetic variants that alter protein structure ~ Exploring the pathogenesis of diseases by deciphering the complexity of alternative splicing~〔*Department of Genomic Function and Diversity*〕

- International collaborative genome analysis that elucidated the genetic background of rheumatoid arthritis by GWAS meta-analysis ~Contributing to implementation of disease prediction using individual genomic information~〔*Department of Genomic Function and Diversity*〕

Department of Structural Biology

Professor Nobutoshi Ito
Associate Professor Nobutaka Numoto
Assistant Professor Yuya Hanazono

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. Structure-function relationship of human-derived B-cell inhibitory factor CD72

The development of autoimmune diseases is largely associated with the production of antibodies against self-antigens, including nucleic acids. We have focused on the inhibitory co-receptor CD72 expressed on B cells and have shown that CD72 specifically binds to the nuclear autoantigen Sm/RNP (U1 snRNP), including RNA, and suppresses the production of autoantibodies to Sm/RNP. We have previously reported the crystal structure of the ligand-binding domain (CTLD) of mouse CD72 and provided the structural basis for the molecular mechanism of specific recognition of Sm/RNP, but the structure of human CD72 was unknown. Suppression of autoantibody production against Sm/RNP by CD72 suppresses the onset of systemic lupus erythematosus (SLE), a typical systemic autoimmune disease, and CD72 polymorphisms in mice and humans are associated with the onset of SLE. Elucidation of the ligand recognition mechanism based on the detailed three-dimensional structure of human CD72 is expected to lead to the development of new therapeutic strategies.

Human CD72-CTLD contains a longer insertion sequence than that of mouse CD72-CTLD (Fig. 1), which is thought to be a long loop region that does not have a rigid structure. Since these random structure regions are disadvantageous for structural analysis, we prepared a human CD72-CTLD mutant lacking this insertion sequence. We have successfully determined the structure of the human CD72-CTLD mutant by X-ray crystallographic analysis. Data were collected at SPring-8, a synchrotron radiation facility in Japan, from a large amount of the crystal clusters by using a high-intensity microfocus X-ray beam and an automatic measurement/analysis system. Comparison with the structure of mouse CD72-CTLD allows us to discuss the molecular mechanism of how CD72 recognizes Sm/RNP (Fig. 1), a nucleic acid-protein complex, and regulates its binding affinity. The electrostatic potentials of the molecular surface of human and mouse CD72-CTLD at the putative ligand-binding sites are quite different, which is thought to have a particularly large effect on the affinity with nucleic acids. The obtained structure and protein-ligand interaction analysis indicated that the insertion loop, which was deleted in the structural analysis, does not significantly affect the recognition of the ligand. In order to obtain more detailed structural information on the molecular recognition mechanism, structural analysis of the complex of CD72-CTLD and Sm/RNP is underway. The goal is to obtain the structural basis for the rational design of novel molecules that regulate CD72 function, which will allow us to establish new therapies for autoimmune diseases that utilize CD72 function.

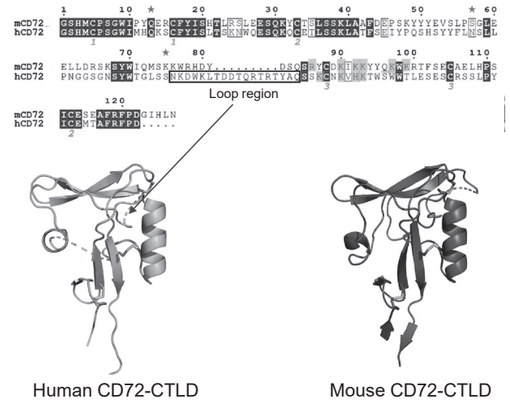

Fig.1 (Upper panel) Amino acid sequence alignment of human and mouse CD72-CTLD. Insertion (loop) region of human CD72-CTLD is highlighted as a box. (Lower panel) Crystal structures of human and mouse CD72-CTLD. Insertion region is indicated as black lines.

This work is performed in collaboration with Professor Takeshi Tsubata at Nihon University.

2. Search for novel ligand compounds for vitamin D receptor (VDR)

Active vitamin D_3 (1α,25-dihydroxyvitamin D_3), which is derived from vitamin D, is a type of steroid hormone that regulates blood calcium levels, regulates immunity, inhibits cell growth, and promotes differentiation. Therefore, while vitamin D derivatives are used to treat bone diseases like rickets and osteoporosis, as well as skin diseases like psoriasis, they may cause hypercalcemia as a side effect. As a result, it is necessary to search for compounds that act on VDR differently than active vitamin D_3. The crystal structure of various novel ligands in complex with the VDR ligand binding domain (VDR-LBD) is being studied to better understand the molecular recognition mechanism and to rationally design more highly functional ligand molecules.

Lithocholic acid has been identified as a second endogenous VDR agonist. Although lithocholic acid itself lacks VDR binding affinity and vitamin D activity, we have previously reported that a lithocholic acid derivative (Dcha-20) is a potent agonist and that its carboxyl group interacts with the ligand binding site of VDR. Recently, it was reported that Dcha-20 has lower glycemic activity than active vitamin D_3, which may be beneficial for clinical use. However, preliminary Dcha-20 pharmacokinetic studies show that it is excreted quickly in mice. Therefore, to clarify the function of the carboxyl group in lithocholic acid, amide derivatives of Dcha-20 were designed, and compounds with N-cyano and N-2-carboxyethyl groups demonstrated potent activity. Among them, we performed the structural analysis of compounds containing N-2-carboxyethyl groups and VDR-LBD. The terminal carboxyl group of Dcha-20 derivative forms a hydrogen bond with the amino acid residue of VDR-LBD, rather than the amide group, implying that the carboxyl group of Dcha-20 is important for both potent vitamin D activity and pharmacokinetic properties. These findings suggest that modification of the terminal polar group of lithocholic acid may create lithocholic acid derivatives with high activity and pharmacokinetic features.

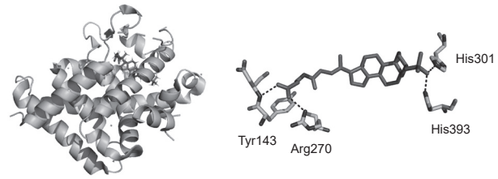

Fig.2 (Left) Crystal structure of VDR-LBD and lithocholic acid derivative complex (Right) Interaction between VDR-LBD and lithocholic acid derivative.

This work is performed in collaboration with Professor Hiroyuki Kagechika at Institute of Biomaterials and Bioengineering, Professor Makoto Makishima at Nihon University, and Associate Professor Aya Tanatani at Ochanomizu 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.

Publications

1. Numoto N., Onoda S., Kawano Y., Okumura H., Baba S., Fukumori Y., Miki K., Ito N. Structures of oxygen dissociation intermediates of 400 kDa V2 hemoglobin provide coarse snapshots of the protein allostery. Biophys. Physicobiol., 19, e190019, 2022.
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Department of Functional Genome Informatics

Professor Itoshi Nikaido, Ph.D.
Associate Professor Yohei Sasagawa, Ph.D.
Assistant Professor Mariko Yamane, Ph.D.

Research Objects

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

Research activities

(1) Expression diversity and activation of genes involved in immunity

The transcription factor NF-κB, which plays an important role in cell fate determination, is involved in the activation of super-enhancers (SEs). However, the biological functions of NF-κB in gene regulation of SEs have not been fully elucidated. We joined a research group led by Dr. Mariko Okada and Dr. Johannes N Wibisana of Laboratory for Cell System, Institute for Protein Research, Osaka University, to study NF-κB-mediated SE activity. We investigated the characteristics of NF-κB-mediated SE activity in anti-IgM-stimulated B cells using RelA fluorescence imaging, single cell transcriptome analysis, and chromatin accessibility analysis. Cell-stimulated nuclear RelA focus formation disappeared in the presence of hexanediol, suggesting an underlying liquid-liquid phase separation process. The acquired SEs induced switch-like expression and enhanced the cell-to-cell variability of the transcriptional response. These properties correlated with the number of cis-regulatory interactions acquired, and switch-like gene induction correlated with the number of NF-κB binding sites in the SEs. This study suggests that NF-κB SEs play an important role in the transcriptional regulation of B cells, probably through the formation of liquid condensates composed of macromolecular interactions.

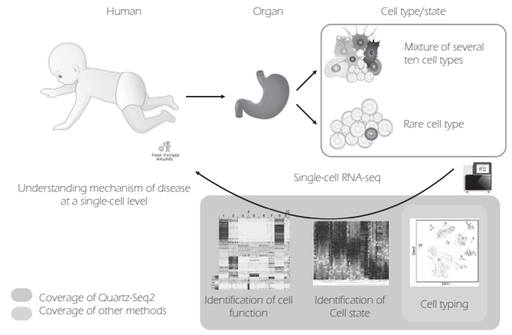
(2) Redefining Throughput in Life Science Measurement

In recent years, technological advances in photonics, electronics, computing, fluidics, robotics, and chemistry have greatly increased the speed and scale of automation and parallelization of life science data acquisition and processing. This has resulted in high-throughput performance in molecular, compound, genetic, and cellular measurements, as well as DNA sequencing. The term “throughput” is widely accepted and constantly used in the biomedical community. High throughput is essential for efficient, reproducible, time-critical, low-cost, and scarce applications.

However, the term is often vaguely defined and not consistently used in different areas of the life sciences. In fact, it is used differently than “throughput,” which is strictly defined in the field of electrical engineering, where throughput theoretically represents the maximum amount of data that can be transmitted or processed in a given amount of time and is quantified in bits per second. In life sciences, throughput depends on non-electrical parameters (e.g., chemical, biological, fluid) and is often non-uniform and time-varying, so the actual throughput achieved can be several orders of magnitude lower than the theoretical throughput. Furthermore, the throughput unit differs among research areas, inhibiting inter-disciplinary collaboration. Therefore, we proposed distinguishing between theoretically achievable and practically achievable throughput in our calculations.

(3) Mouse models of autism and immune abnormalities

An international research group led by Professor Toru Uchisho, Researcher Chia-Wen Lin at Kobe University Graduate School of Medicine, and our group has revealed that idiopathic autism is caused by epigenetic abnormalities in hematopoietic cells during fetal development, resulting in immune abnormalities in the brain and gut. The research group has revealed that the cause of idiopathic autism is an epigenetic abnormality of hematopoietic cells in the fetus, resulting in immune abnormalities in the brain and intestines. As the classification of the pathophysiology of autism progresses, it is expected that new treatment strategies for autism and other neurodevelopmental disorders will be created in the future.



Moving in: Minoru Yano (Technical staff), Ryoko Seki (Technical staff), Atsumi Soma (Technical staff), Yoshimi Iwayama (Guest lecturer)
Moving out: none

Research achievements

1. Johannes N Wibisana, Takehiko Inaba, Hisaaki Shinohara, Noriko Yumoto, Tetsutaro Hayashi, Mana Umeda, Masashi Ebisawa, Itoshi Nikaido, Yasushi Sako, Mariko Okada. Enhanced transcriptional heterogeneity mediated by NF-κB super-enhancers. PLoS genetics. 2022.
2. Maik Herbig, Akihiro Isozaki, Dino Di Carlo, Jochen Guck, Nao Nitta, Robert Damoiseaux, Shogo Kamikawaji, Eigo Suyama, Hirofumi Shintaku, Angela Ruohao Wu, Itoshi Nikaido, Keisuke Goda. Best practices for reporting throughput in biomedical research. Nature methods. 2022.
3. Chia-Wen Lin, Dian E Septyaningtrias, Hsu-Wen Chao, Mikiko Konda, Koji Atarashi, Kozue Takeshita, Kota Tamada, Jun Nomura, Yohei Sasagawa, Kaori Tanaka, Itoshi Nikaido, Kenya Honda, Thomas J McHugh, Toru Takumi. A common epigenetic mechanism across different cellular origins underlies systemic immune dysregulation in an idiopathic autism mouse model. Molecular psychiatry. 2022.
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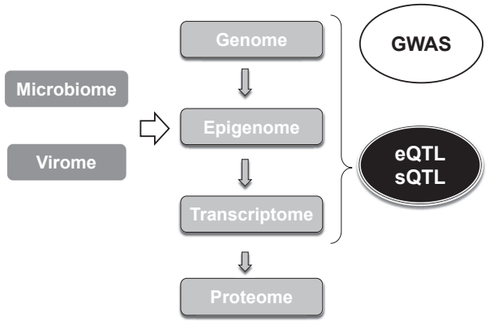
Department of Genomic Function and Diversity

Professor Yuta Kochi
Associate professor Satomi Mitsuhashi
Nao Nishida (~ Jan. 2023)
Assistant professor Mahoko Ueda

Research objectives

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



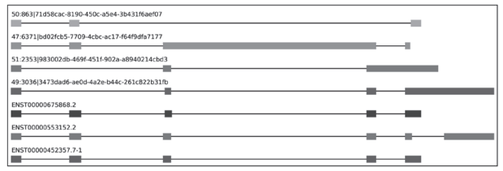
Research activities

1. Integration of GWAS and eQTL/sQTL studies

Majority of GWAS loci identified in complex traits are now considered to be eQTL or sQTL where genetic variants regulate expression levels of genes or alternate splicing. Therefore, to interpret the results of GWAS for dissecting the mechanism of disease, it is essential to integrate the results of GWAS and QTL studies. We have performed eQTL studies for immune cell subsets to establish eQTL catalog in Japanese（*Nat Genet 2017, Cell* 2021）. By combining those eQTL data with disease GWAS data, we have successfully identified many disease-associated eQTL.

First, we developed a method, called integrated-isoform ratio QTL (i²r-QTL) analysis, for comprehensively clarifying sQTL that change the protein sequences (*Nat Commun* 2022). In addition, we performed comprehensive expression analysis by long-read sequencing (long-read RNA-seq) for 28 subsets of immune cells to create an Immune Isoform Atlas (*bioRxiv* 2022). This atlas will advance the elucidation of disease mechanism for various immune-related diseases.

Splicing isoforms of OAS1, a risk gene for the severe COVID-19



2. Search for disease susceptibility genes

From the results of GWASs conducted so far, it is evident that there are differences in genetic factors among different populations. Our international joint research group (Rheumatoid Arthritis Consortium International: RACI) identified 34 novel loci for rheumatoid arthritis by performing a meta-analysis of GWAS data of various populations around the world (*Nat Genet* 2022). In addition, we launched a genome project with the aim of enabling diagnosis and prognosis of preclinical phase of rheumatoid arthritis patients using whole-genome sequencing (AMED, Immuno-Allergic Disease Project).

3. Analysis of 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. Polygenic risk score (PRS) is one of promising approaches, and we showed that PRS constructed by the data from GWAS for rheumatoid arthritis can predict radiographic progression in patients (*Arthritis Rheumatol* 2022). We will further improve this prediction model by integrating various omics data such as GWAS, eQTL, and sQTL to realize precision medicine in clinic.

Personnel change

Joined : Nao Nishida (Associate professor), Nomi Iwasa (Secretary), Eri Ito (Technical staff)

Publications

Original articles

1. Honda S, Ikari K, Yano K, Terao C, Tanaka E, Harigai M, Kochi Y. Polygenic risk scores are associated with radiographic progression in patients with rheumatoid arthritis. ***Arthritis Rheumatol***. 2022, AOP.
2. Yin X, Kim K, Suetsugu H, Bang SY, Wen L, et al Meta-analysis of 208370 East Asians identifies 113 susceptibility loci for systemic lupus erythematosus. ***Ann Rheum Dis***. 2021;80(5):632-40.
3. Tanaka N, Koido M, Suzuki A, Otomo N, Suetsugu H, Kochi Y, et al. Eight novel susceptibility loci and putative causal variants in atopic dermatitis. ***J Allergy Clin Immunol***. 2021;148(5):1293-306.
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5. Ota M, Nagafuchi Y, Hatano H, Ishigaki K, Terao C, et al. Dynamic landscape of immune cell-specific gene regulation in immune-mediated diseases. ***Cell***. 2021;184(11):3006-21 e17.
6. Mitsuhashi S, Nakagawa S, Sasaki-Honda M, Sakurai H, Frith MC, Mitsuhashi H. Nanopore direct RNA sequencing detects DUX4-activated repeats and isoforms in human muscle cells. ***Hum Mol Genet***. 2021;30(7):552-63.
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8. Bing N, Zhou H, Chen X, Hirose T, Kochi Y, et al. Contribution of a European-Prevalent Variant near CD83 and an East Asian-Prevalent Variant near IL17RB to Herpes Zoster Risk in Tofacitinib Treatment: Results of Genome-Wide Association Study Meta-Analyses. ***Arthritis Rheumatol***. 2021;73(7):1155-66.

Review articles

1. Kochi Y. Genetics of rheumatoid arthritis (in Japanese). ***Nihon Rinsho***. 80(4)41-45, 20221.
2. Kochi Y. Genetics of systemic lupus erythematosus (in Japanese). ***Nihon Rinsho***. 80(5)757-762, 2022

Joint Research Division

Laboratory for Integrated Research Projects on Intractable Diseases

Advanced Technology Laboratories

Joint Research Division, Department of Precision Health

Associate Professor, Joint Research Division — **Takahiro Adachi**
(Concurrent Post) Professor, Department of Developmental and Regenerative Biology — **Hiroshi Nishina**
Technical Assistant — **Megumi Tobita**
Joint Researcher — **Takuto Hayashi**

Atopy and developmental disorders in children, and lifestyle-related diseases are increasing, and dementia is becoming a social problem. It has been found that chronic inflammation caused by environmental factors other than genetic factors is a predisposition to diseases, and it has been pointed out that there is a correlation between mutual diseases. If we can detect the predisposition of diseases, we will be less burdened and can prevent various diseases, resulting in extending healthy life. Recently, "prevention/treatment of predisposition of diseases" targeting before showing signs of illness (pre-illness) has been touted. For that purpose, it is necessary to monitor biological information with high sensitivity and to develop a "prevention/treatment" method for the predisposition of diseases. Therefore, we are working on research aimed at developing preventive and therapeutic methods that are less burdensome to us by detecting slight abnormalities in the body more quickly and easily.

1. Analysis of immune response

We have established a cell lineage-specific calcium biosensor (YC3.60) mouse that can monitor not only the dynamics of immune cells but also activation *in vivo*, and established a 6D (x, y, z, time, Ca^{2+} signaling, cell labeling) intravital imaging system (Highlight). Using this system, it is possible to visualize the activation and differentiation of immune cells in the living body in real-time. We have also found that bioimaging focusing on intracellular Ca^{2+} signaling using these mice can detect the predisposition of diseases at a very early phase before developing the pathological disorders (Figure 1). We are trying to further develop this system and to clarify the onset of various diseases such as allergies, viral infections, and autoimmune diseases, and the detailed elucidation of the events occurring in the process of pathological progress.

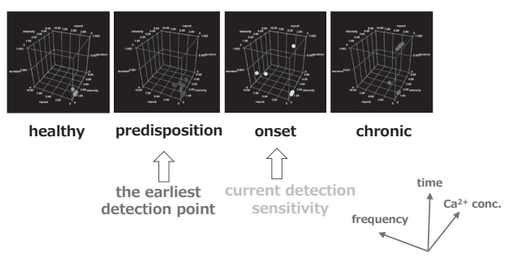


Figure 1. Ultrasensitive health monitoring system
Intravital imaging of Ca^{2+} signaling in mmune cells using lineage-specific calcium biosensor mice allow to detect subtle predisposing abnormalities prior to pathogenesis, based on normal conditions. With this system, the progression from the onset of disease to chronic disease can also be detected with high sensitivity, and the health status of living organisms can be monitored.

2. Analysis of intestinal sensing network and organ linkage

The immune system, peripheral nervous system, and endocrine system are concentrated in the intestinal tract, and information is directly exchanged by the brain-gut axis. To clarify how foods, medicines, etc. orally ingested are recognized in the intestinal tract, we have established intravital imaging of the enteric nervous system, immune cells, or enteric epithelial cells in a cell-specific manner. Based on these established technologies, we are trying to clarify the crosstalk such as the gut-brain and the gut-skin mediated by orally administrated food and medicine.

3. Establishment of prevention/treatment methods for predisposition of diseases and methods to increase robustness for health

We aim to develop foods and medicines that target the predisposition of diseases, as well as foods and medicines that enhance physical and mental health. We evaluate the effects of foods, natural products, and their components on the immune system, nervous system, and intestinal epithelium including endocrine by the established system by us. We are developing preventive/therapeutic methods and robustness acquisition methods using a model mouse system that has an abnormality or predisposition of diseases in the intestinal/skin barrier function and a dietary obese mouse model system.

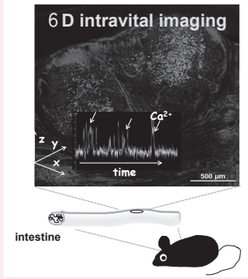
4. Clinical application for monitoring of predisposition of diseases

We aim to establish a method for detecting slight changes (predisposition of diseases) before the onset of diseases and try to develop foods and medicine to prevent and treat these disorders. Based on these basic researches, we are aiming to develop a device that can easily measure slight abnormalities that predispose to diseases such as lifestyle-related diseases and developmental disorders in humans for clinical application.

Highlight

- Generation of the conditional calcium biosensor mice
- 6D (x, y, z, time, ca^{2+} signaling, cell labeling) intravital imaging of immune cells
- Visualization system using *in vivo* imaging of intestinal (epithelial, immune, nerve) sensing by food signals
- Establishment of a dual real-time visualization system for food signals in the gut-brain axis *in vivo*

(Received an academic award from the Japanese Association for Food Immunology in 2022)



6D intravital imaging with conditional calcium biosensor mice

We generated a conditional calcium biosensor (Yellow Cameleon3.60) mouse line and established a 6D bioimaging system that can measure six parameters: x, y, z, time, cell labeling, and ca^{2+} signaling.

Publications

Takeuchi, Y., Fukunaga, M., Iwatani, S., Miyanaga, K., Adachi , T., Yamamoto, N. Release of an anti-anxiety peptide in casein hydrolysate with *Aspergillus oryzae* protease. Food Funct. 13(20):10449-10460. 2022.

Kotake, K., Kumazawa, T, Adachi, T. Long-term administration of *Tetragenococcus halophilus* No. 1 over generations affects the immune system of mice. PLoS One, 17(4):e0267473. 2022.

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Laboratory for Integrated Research Projects on Intractable Diseases

Basic and applied research using a novel human squamous cell carcinoma organoid library

Principal Researcher　Toshiaki OHTEKI
Research collaborators　Taku SATO, Yusuke KINUGASA, Hiroyuki HARADA, Yoshiyuki MORI, Akinori MIURA

Research Outcome

Tongue cancer accounts for approximately 60% of all oral cancers, and the five-year survival rate is extremely low at 42% for advanced cancer. Postoperative curative treatment with anticancer agents and radiation is used, but recurrence is seen in 24-48% of patients. Similarly, esophageal squamous cell carcinoma, which is characteristic of Asian countries including Japan, has a high recurrence rate of 30-50% after curative treatment. Using our original method, we have successfully constructed human tongue and esophageal cancer organoid libraries (28 tongue cancer organoids and 24 esophageal cancer organoids) (Figure 1). These included several cancer organoid cases (4 tongue cancer organoids and 6 esophageal cancer organoids) that were resistant to the anticancer drugs used in current clinical treatment. These are being used to promote the following studies.

1) Elucidation of the mechanism of acquisition of anticancer drug resistance using squamous cell carcinoma organoids.

With the aim of elucidating the molecular basis of chemotherapeutic drug resistance by identifying cancer diversity among patients, we compared the gene expression profiles of chemotherapy-resistant and chemotherapy-sensitive tongue cancer organoid lines and identified biological pathways that are significantly activated or inactivated in the former. Inhibition of specific pathways activated in chemotherapy-resistant tongue cancer organoid lines by small molecules significantly reduced their survival, while inactivation of specific pathways activated in chemotherapy-sensitive tongue cancer organoid lines increased their resistance to chemotherapeutic agents.

On the other hand, for esophageal squamous cell carcinoma organoids, gene expression analysis also identified a pathway that is likely to be involved in chemotherapeutic drug resistance. We are currently generating chemotherapeutic drug-resistant cancer organoid lines in which this transcription factor, which plays a central role in chemotherapeutic drug resistance, has been knocked down.

2) Discovery of existing drugs targeting anti-cancer drug-resistant cancers

We targeted drugs (small molecule compounds) that have already been approved by the FDA, PMDA. As a result, we succeeded in identifying several drugs that could significantly inhibit the growth and survival of each drug-resistant tongue cancer organoid strain and esophageal squamous cell carcinoma organoid strain more than the chemotherapeutic agents used in actual clinical practice. We plan to generate target molecule knockdown strains of these drugs to verify their effects on survival and proliferation and to test their in vivo effects using the cancer organoid PDX model.

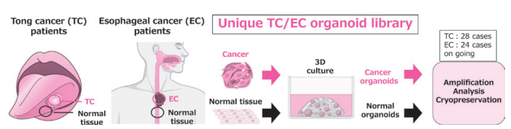


Fig.1 Establishment of human tongue cancer/esophageal cancer organoid library

Analysis of splicing isoforms in intractable immune diseases based on genomic information

Project leader　Yuta Kochi
Collaborators　Kensuke Yamaguchi, Takashi Satoh, Shinsuke Yasuda, Satomi Mitsuhashi

Summary

In this project, experts in genomic medicine, immunology, and rheumatology will conduct cross-disciplinary research focusing on complex immunological diseases such as autoimmune diseases and severe COVID-19. We make a special focus on the functional dissection of splicing isoforms and evaluate them as drug targets.

Research Outcome

1. Development of sQTL analysis method

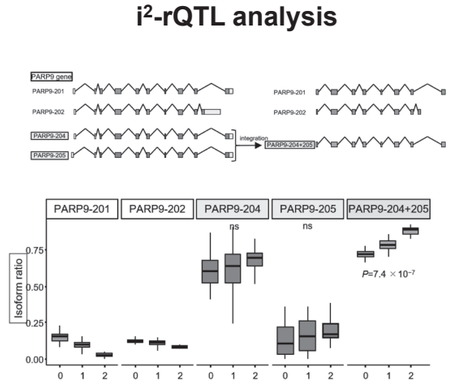
A genetic variant that affects alternative splicing is defined as a splicing quantitative trait locus (sQTL). While various isoforms are generated by alternative splicing, we developed a method, called integrated-isoform ratio QTL (i^2r-QTL) analysis, for comprehensively clarifying sQTL that change the protein sequences (*Nat Commun* 2022).

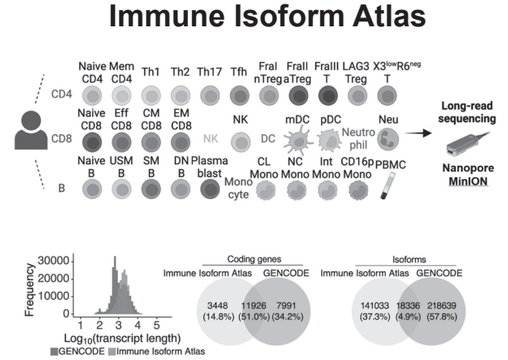
2. Analysis of disease associated isoform

Our international joint research group (Rheumatoid Arthritis Consortium International: RACI) identified 126 loci for rheumatoid arthritis by performing a meta-analysis of GWAS data of various populations around the world. Of these, we identified an isoform in PADI4 gene, which lacked its C-terminal domain, by long-read RNA-seq and revealed that the decreased expression of this isoform caused the disease (*Nat Genet* 2022).

3. Establishment of Immune Isoform Atlas

We performed comprehensive expression analysis by long-read sequencing (long-read RNA-seq) for 28 subsets of immune cells to create an Immune Isoform Atlas (*bioRxiv* 2022). This atlas will advance the elucidation of disease mechanism for various immune-related diseases.





Publications

1. Yamaguchi K, Ishigaki K, Suzuki A, Tsuchida Y, Tsuchiya H, et al. Splicing QTL analysis focusing on coding sequences reveals mechanisms for disease susceptibility loci. ***Nat Commun***. 13. 4659, 2022.
2. Ishigaki K, Sakaue S, Terao C, Luo Y, Sonehara K, et al. Multi-ancestry genome-wide association analyses identify novel genetic mechanisms in rheumatoid arthritis. ***Nat Genet***. 54. 1640-51, 2022.
3. Inamo J, Suzuki A, Ueda M, Yamaguchi K, Nishida H, et al. Immune Isoform Atlas: Landscape of alternative splicing in human immune cells. ***bioRxiv*** 2022.

Elucidation of pathogenic mechanism of Parkinson's disease and drug development research.

Project Associate Professor/Project Leader Satoru TORII
Professor Shigeomi SHIMIZU
Professor Takamitsu HOSOYA

Summary

Parkinson's disease is a neurological intractable disease that often develops in people in their 50s and 60s, with progressive symptoms such as tremors in the limbs. More than 140,000 people suffer from the disease in Japan, but no fundamental treatment exists to date. Degeneration and loss of dopaminergic neurons in substantia nigra of midbrain are known to be the cause of the disease. In this project, the pathogenic mechanisms of this disease are being elucidated and new drugs are being developed. Specifically, (1) The pathogenic mechanism of the disease caused by mutations in the PARK22 gene will be elucidated using cultured cells and model mice. (2) We will identify compounds that can alleviate the disease from low-molecular compounds and conduct drug discovery and development research. (3) We will apply our findings to other familial and sporadic Parkinson's disease to gain an integrated understanding. The results of this year's research are as follows.

Research outcome

Result1 : Increased phosphorylated synuclein and aggresome/fibrils were observed in a PARK22 mutant expressing cells. Moreover, protein kinases which binds to the mutant protein was identified. Knock-in mice with the PARK22 gene mutation have been generated and analyzed. Abnormalities similar to those in cultured cells were found in the midbrain substantia nigra of mice.

Result2 : Compounds which can alleviate the abnormalities caused by the PARK22 gene mutation were identified. The compounds improved locomotor function in knock-in mice. A more effective compound was found from derivatives of these compounds.

Result3 : We have analyzed whether the PARK22 gene is also associated with the development of sporadic Parkinson's disease.

Publications

[Original Paper]

1. Yanagi T, Kikuchi H, Susa K, Takahashi N, Bamba H, Suzuki T, Nakano Y, Fujiki T, Mori Y, Ando F, Mandai S, Mori T, Takeuchi K, Honda S, Torii S, Shimizu S, Rai T, Uchida S, Sohara E. Absence of ULK1 decreases AMPK activity in the kidney, leading to chronic kidney disease progression. ***Genes to Cells***, in press (2022).

Advanced Technology Laboratories

Genome Laboratory

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

Followings are the achievements in 2022.

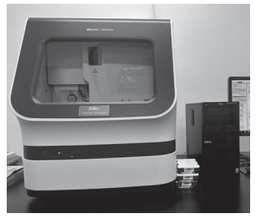
1. Sequencing analyses

A total of 10,714 samples from 1,211 researchers were sequenced in the year of 2022. Among them 6,354 (60%) 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 7 runs were done in the year of 2022. Library preparation service for next generation sequencing has been started in 2015 and 50 samples were done in the year of 2022.

2. Equipment under the management of the Genome Laboratory.

DNA sequencer (ABI3130xl) × 1, DNA sequencer (ABI3500xl) × 1,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.


Ion S5

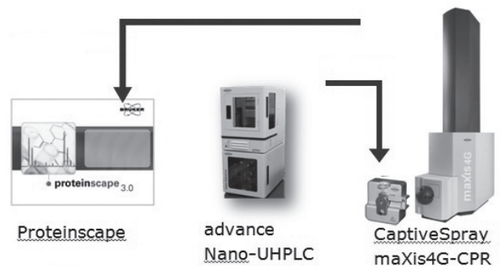

Capillary Sequencer 3500 xl

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

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

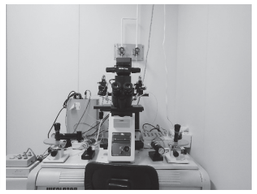
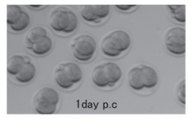


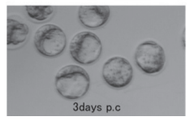
maxis-4G-CPRsystem Bruker Japan

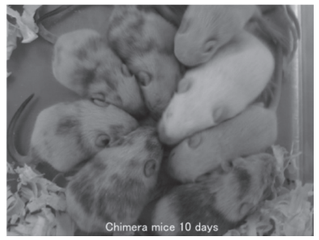
Laboratory of Genome Editing for Biomedical Research

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…5/13 (Carl Zeiss)
Laser Capture Microdissection…12/5 (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 Research Core of this university has started from 2021. Now the Laboratory is equipped with basic and state-of-the-art research instruments, including cell sorters (MoFlo XDP and newly equipped FACSAria Fusion as of December 1, 2022), 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 two Professors and two Associate and Junior 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 234 in the year of 2022.

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 with the related laws and regulations. This year, we received orders from a domestic university (5 cell lines) and a laboratory in campus (1 cell line), and distributed cells to them. We establish EB-virus transformed cell lines 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 (Fig.1). We are constantly receiving requests for this service from our own hospital as well as other research institutes. This year, we received a new request from a domestic university. We also undertake tests for mycoplasma contamination. We continued the preservation service for the biological samples using a large liquid nitrogen tank (Fig.2), which was requested by many laboratories on campus.

In addition, a company used the service of mycoplasma test in this year in the agreement with the Tokyo Metropolitan Government regarding the use of university-owned equipment that contributes to drug discovery and medical open innovation.

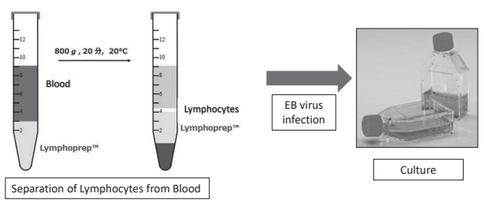


Fig. 1 Establishing Human B Lymphoblastic cell Lines using EBV

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

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.

Single-cell Omics Laboratory

Professor Itoshi Nikaido, Ph.D.
Associate Professor Yohei Sasagawa, Ph.D.
Technical Staff Minoru Yano, Ph.D.

The Single Cell Omics Laboratory is developing single cell level measurement and data analysis techniques. These technologies are also used to promote collaborative research within the university. The Single Cell Omics Laboratory is operated in cooperation with the Department of Functional Genome Informatics and the Research Platform Cluster of the Integrated Research Organization. It also contributes to the project as the core of single-cell omics analysis of the High-Depth Omics Medical Research Center Project promoted by the Research Institute for Intractable Diseases.

We have successfully developed Quartz-Seq2, the world's highest performance single-cell RNA sequencing method (Sasagawa Y. et al. Genome Biol. 2013, 2018, Mereu E. et al. Nature Biotech. 2020). Using this technology, all RNA types and numbers in a single cell can be accurately measured, and Quartz-Seq2 can be used to characterize all cell types in an organ or tissue. When cell characteristics in organs and tissues where disease has occurred are investigated, the causes of disease can be elucidated, and drug discovery can be conducted.

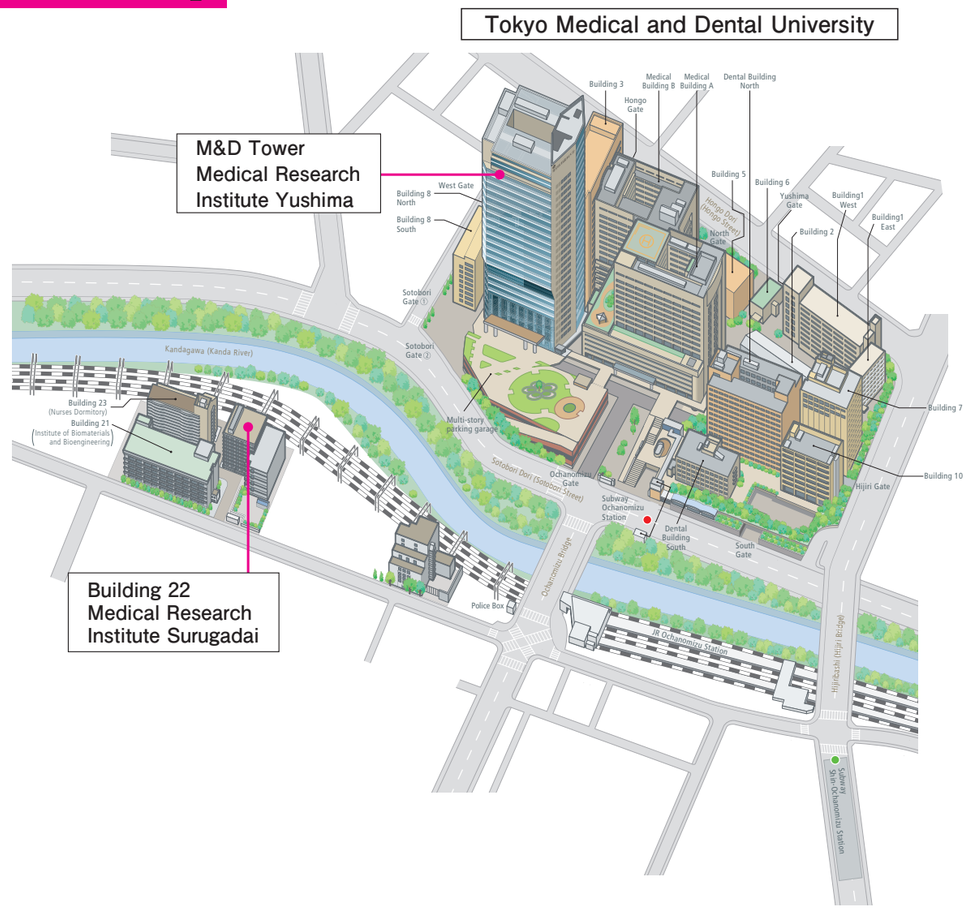
In FY2022, four intra- and two external collaborations (one of which was with a company) were conducted, 20,000 samples were sequenced, and two papers were published.

Research achievements

1. Johannes N Wibisana, Takehiko Inaba, Hisaaki Shinohara, Noriko Yumoto, Tetsutaro Hayashi, Mana Umeda, Masashi Ebisawa, Itoshi Nikaido, Yasushi Sako, Mariko Okada. Enhanced transcriptional heterogeneity mediated by NF-κB super-enhancers. PLoS genetics. 2022.

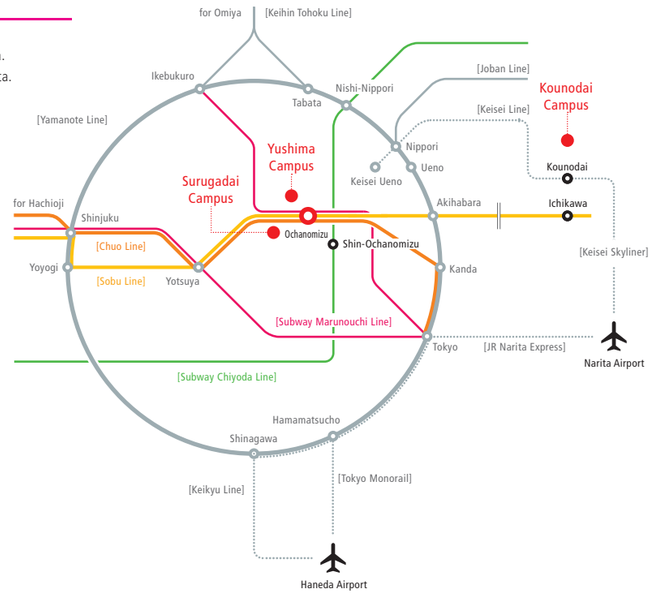
2. Chia-Wen Lin, Dian E Septyaningtrias, Hsu-Wen Chao, Mikiko Konda, Koji Atarashi, Kozue Takeshita, Kota Tamada, Jun Nomura, Yohei Sasagawa, Kaori Tanaka, Itoshi Nikaido, Kenya Honda, Thomas J McHugh, Toru Takumi. A common epigenetic mechanism across different cellular origins underlies systemic immune dysregulation in an idiopathic autism mouse model. Molecular psychiatry. 2022.

Access Map



Nearest Stations

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



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