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ANNUAL REPORT 2024

Tokyo Medical and Dental University

Medical Research Institute / Tokyo Medical and Dental University

Annual Report 2024

2024

Annual Report

Medical Research Institute

Tokyo Medical and Dental University

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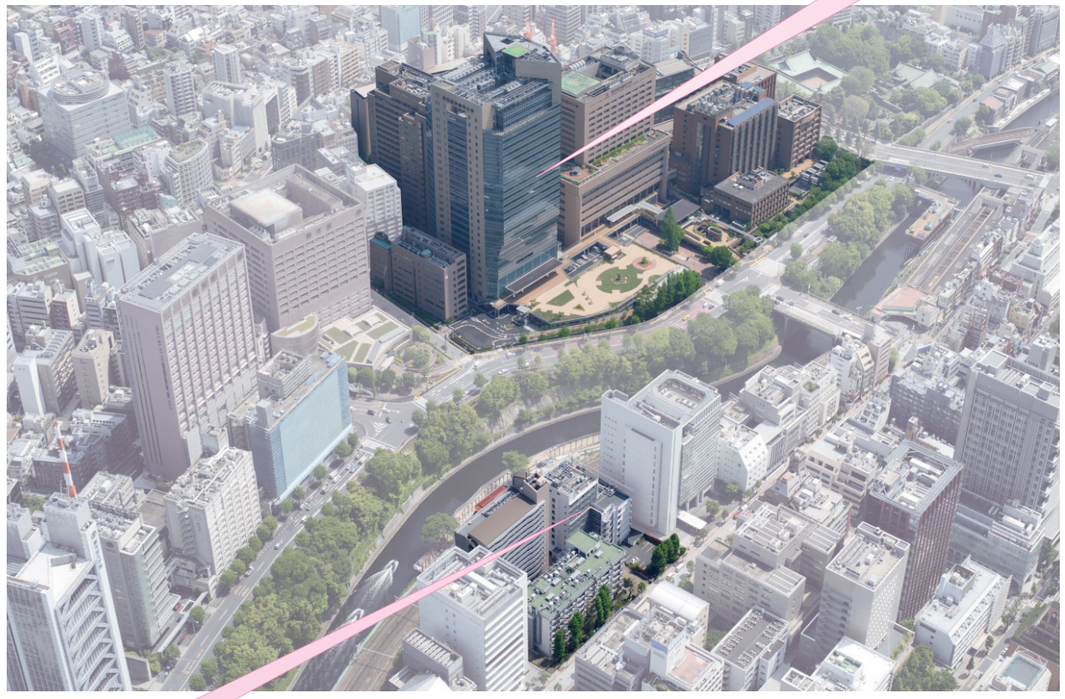
Medical Research Institute

Yushima Area

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

Medical Research Institute

Department of Medical Chemistry, Department of Biochemical Pathophysiology, Department of Developmental and Regenerative Biology, Department of Molecular Cell Biology, Department of Stem Cell Regulation, Department of Homeostatic Medicine, Department of Biomolecular Pathogenesis, Department of Biodefense Research, Department of Neuropathology, Department of Molecular Neuroscience, Department of Pathological Cell Biology, Department of Neuroinflammation and Repair, Department of Structural Biology, Department of Functional Genome Informatics, Department of Genomic Function and Diversity, Department of Computational and Systems Biology, Department of Advanced Nanomedical Engineering, Administration Office



Surugadai Area

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

Medical Research Institute

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

Medical Research Institute

Institute of Science Tokyo will be established on October 1, 2024, following the merger between Tokyo Medical and Dental University and Tokyo Institute of Technology.

- Director: Hiroshi Nishina
  - Faculty Meeting
- Deputy Director: Yuta Kochi, Takehiko Sasaki
- Research Divisions
  - Division of Visionary Life Science
    - Department of Medical Chemistry — Professor — Katsumori Segawa
    - Department of Biochemical Pathophysiology — Professor — Takehiko Sasaki
    - Department of Developmental and Regenerative Biology — Professor — Hiroshi Nishina
    - Department of Molecular Cell Biology — Professor — Hiroshi Shibuya
    - Department of Stem Cell Regulation — Professor — Tetsuya Taga
    - Department of Homeostatic Medicine — Professor — Fumiko Toyoshima
    - Department of Molecular Epidemiology — (under consideration)
    - 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 Immune Regulation — Professor — Noriko Komatsu
    - Department of Neuroinflammation and Repair — Professor — Takashi Shichita
    - Department of Molecular Neuroscience — (under consideration)
    - 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 Computational Drug Discovery and Design — Professor — Ryuichiro Ishitani
    - Department of Computational and Systems Biology — Professor — Teppei Shimamura
    - Department of Advanced Nanomedical Engineering — Professor — Satoshi Uchida
    - Department of Bio-informational Pharmacology — (under consideration)
    - Frontier Research Unit
- Joint Research Departments
  - Department of Precision Health
- Division of Integrative Research
  - Division of Biosystem Generation
  - Division of Pathogenetic Regulation
- Young Researcher Development Laboratory
- Intractable Disease Integrated Research Laboratory
- COVID-19 Integrated Research Laboratory
- Laboratory for Joint Usage/Research Center & Research System Project "Overcoming multi-level stress disease"
- Advanced Technology Laboratory
  - Genome Laboratory
  - Laboratory of Cytometry and Proteome
  - Laboratory of Genome Editing for Biomedical Research
  - Laboratory of Anatomy and Cell Function
  - Bioresource Laboratory
  - Stem Cell Laboratory
- Medical Research Center Initiative for High Depth Omics
  - Laboratory for Structure Analysis
  - information Support Office
  - 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.

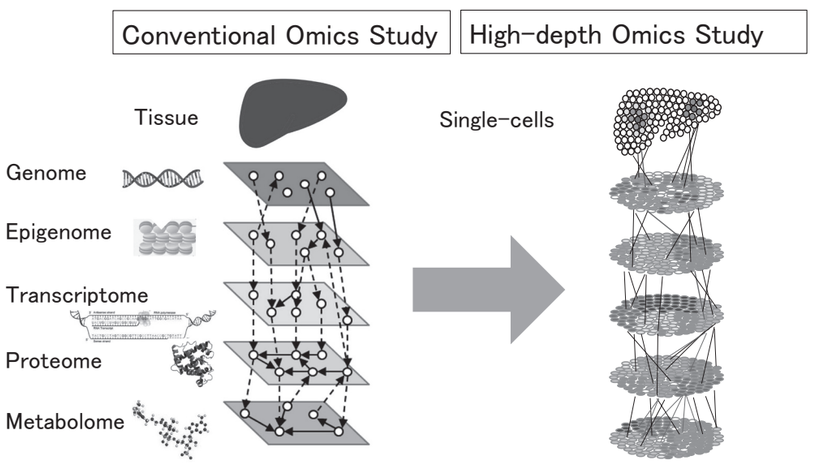
Project Background

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 FY2023

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 long-read sequencer's installation, and the carrying out research collaborations. We hold four meetings of management committee for the laboratory. In addition, four joint research projects were conducted within and nine outside the university (two was with a company), and eight papers were published.

Events

The 2nd Symposium of the High-Depth Omics Medical Research
Date: October 5-6, 2023
Location: TMDU

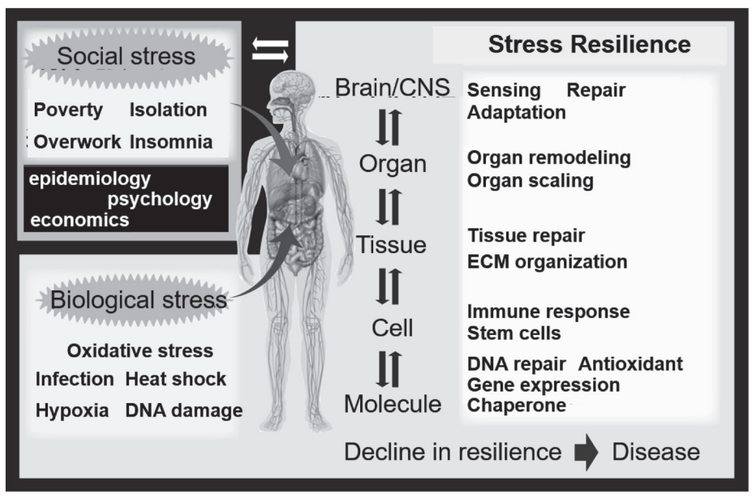
Joint Usage/Research System Project
Coalition of Universities for Research Excellence Program (CURE)
Interdisciplinary Research Hub “Overcoming Muti-level Stress Diseases”

The Medical Research Institute at Tokyo Medical and Dental University (TMDU) was selected for the Joint Usage/Research System Project CURE Program in 2023, and started the Interdisciplinary Research Hub “Overcoming Multilevel Stress Diseases” in collaboration with Tokyo Metropolitan Institute of Medical Science and National Center of Neurology and Psychiatry. This project aims to elucidate the etiological and pathological mechanisms of disorders caused by biological and social stress, from the genes to human society, and to develop diagnostic, preventive, and therapeutic methods. The aim is to provide a basis for new medical treatments and proposals to improve human health in a stressful society.

Purpose of the Project

Living organisms are equipped with mechanisms to sense and adapt to multiple environmental stresses. This flexibility in living organisms is called “stress resilience” and has been studied as a biological strategy that enables organisms to survive in diverse environments. On the other hand, in modern human society, not only biological stresses from nature, but also social stresses that are unique to humans and that have not been faced by living organisms in the past, are spreading rapidly. Various diseases caused by social stress, such as overwork, lack of exercise and sleep, and family and educational environments, have become a global problem. To understand stress resilience as an individual human being and the disease mechanisms caused by its deterioration, it is necessary to clarify the link between stress resilience mechanisms in peripheral tissues and social stress sensing mechanisms in the brain and nervous system.

In this project, the Medical Research Institute at TMDU, which has promoted multidisciplinary and basic research on biological stress diseases, and the Tokyo Metropolitan Institute of Medical Science and the National Center of Neurology and Psychiatry, which have the largest resources on psychiatric and neurological diseases in Japan and have promoted the development of diagnostic, therapeutic and preventive methods and cohort studies, will form a collaborative research hub. This research hub aims to expand conventional biological stress research into contemporary social-type stress research. Through collaborative research and symposia with national and international researchers, we aim to raise the national level of interdisciplinary research in the field of multilevel stress disorders. We aim to educate and train the young researchers who will conduct multi-level stress research, and provide the basis for proposing for new medical and administrative policies that will contribute to the improvement of human health in stressful society.



Activities in 2023

・Established the Interdisciplinary Research Hub Committee at the Medical Research Institute.

・Project participants from each institution were finalized.

・The public resources held by each organization have been organized and shared, and a system has been established to allow access to them through the Hub website.

・A support lab was established within the Medical Research Institute to promote collaboration among the three institutions.

・Open recruitment of project assistant professors and technical assistants to promote this project has begun.

・Three proposals were selected through an open call for grant-funded stress research targeting young researchers at the institute.

Division of Visionary Life Science

【Aim and Scope】

The Division of Visionary Life Sciences 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. The significant research achievements of our division this year are as follows:

【Medical Chemistry】

- Identification of a novel lipid translocase that controls plasma membrane lipid distribution
- Cryo-EM structures of the identified lipid translocase has been determined

【Biochemical Pathophysiology】

- Discovery of changes in the phosphoinositide profile of mouse skeletal muscles with aging
- Identification of a phospholipid metabolizing enzyme involved in the onset of liver cancer

【Developmental and Regenerative Biology】

- Discovery of a novel factor essential for mammalian eye development

【Molecular Cell Biology】

- GID molecules are involved in Wnt signaling regulation via degradation of β-catenin.

【Stem Cell Regulation】

- Development of niche-mimicking polymers for neural stem cell maintenance
- Identification of a novel gene, Rasip1, that contributes to the proliferation and maintenance of hematopoietic stem cells at the embryonic stage
- Niche-mimicking polymer hydrogel-based identification of molecular targets for pancreatic cancer stem cells

【Homeostatic Medicine】

- Elucidating the role of mid-gestational periportal hepatocyte proliferation in preventing gestation diabetes

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. Research on the self-tolerance mechanisms of innate immune cells is also being advanced.

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. This suggests that the constitutive translocation of PtdCho in the plasma membrane could be detrimental to human subjects.

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. 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°C and 15°C, 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°C, and the asymmetric distribution took 30-60 minutes at 37°C 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.

On the other hand, these quadruple knockout cells retained the asymmetric distribution of PtdSer at the plasma membrane, suggesting that the PtdSer asymmetry can be established via a pathway independent of flippases in the plasma membrane and endosomes.

3. Identification of novel phospholipid translocases

Based on our previous research, the existence of two types of phospholipid translocases and pathways has been anticipated. One involves molecules that control the distribution of PtdCho in the plasma membrane, while the other involves the establishment mechanism of PtdSer asymmetric distribution, independent of flippases in the cell membrane and endosomes. This year, we attempted to identify these molecules. As previously mentioned (1), constitutive translocation of PtdCho by the ATP11A-Q84E mutant is harmful to both cells and human subjects. Using this unique system, we tried to identify molecules involved in the translocation of PtdCho in the plasma membrane. Genes of cells expressing ATP11A-Q84E were randomly knocked out using the CRISPR-Cas9 system, and cells with significantly increased the flipping rates of PtdCho by the Q84E mutant at the plasma membrane were recovered using a cell sorter. Analysis of the genes disrupted in these mutant cells using deep sequencing successfully identified a novel gene involved in the translocation of PtdCho at the plasma membrane (Miyata et al, in revision). This molecule alters its structure in response to changes in membrane curvature and tension associated with changes in composition of membrane phospholipids, thereby translocating PtdCho (Miyata et al, in revision). Furthermore, pro-B cell lines lacking the identified gene showed a decrease in PtdCho in the outer leaflet of the plasma membrane, accompanied by an increase in SM. This result suggests that the identified molecule controls the distribution of PtdCho and SM in the plasma membrane. Currently, we are advancing the analysis of the detailed molecular mechanism to understand when and how the molecule regulates the distribution of PtdCho and SM in cells. As mentioned earlier (2), when quadruple knockout cells lacking flippases in the plasma membrane and endosomes expose PtdSer on the cell surface, they continue to expose PtdSer for several hours thereafter. However, under steady-state conditions, quadruple knockout cells maintained the asymmetric distribution of PtdSer in the plasma membrane. Therefore, we randomly disrupted genes in quadruple knockout cells using the CRISPR-Cas9 system and recovered cells that exposed PtdSer on the cell membrane under steady-state conditions using a cell sorter. Analysis of the genes disrupted in these mutant cells identified novel genes involved in the asymmetric distribution of PtdSer. This molecule is a multi-pass membrane protein localized to the endoplasmic reticulum. Currently, we are advancing the analysis of the molecular mechanism to understand how the molecule in the endoplasmic reticulum controls the asymmetric distribution of the plasma membrane PtdSer.

4. Analysis of the self-tolerance mechanisms in innate immune cells

In addition to investigating phospholipid translocases, our research extends to the field of innate immunity. Innate immune cells, such as macrophages, employ various molecules, including pattern recognition receptors, to discern between self and non-self biomolecules and mount specific responses to non-self entities. However, recent findings indicate that these molecules, initially recognized as “non-self,” may also interact with “self” molecules in diverse contexts and degrees. In our bodies, macrophages continually engulf substantial quantities of deceased cells and diverse vesicles, processing them within lysosomes without eliciting immune reactions. This suggests a potential mechanism of tolerance towards self molecules. Currently, we have established a microglial cell line with stable expression of a type I interferon reporter and are progressing with the investigation of self-tolerance mechanisms.

Personnel change

Transfers In:

Yu Shiraki (Short-term Exchange Student)
Aya Nagata (Short-term Exchange Student)
Megumi Nishimura (Short-term Exchange Student)

Department of Biochemical Pathophysiology

Professor	Takehiko Sasaki
Associate Professor	Junko Sasaki
Assistant Professor	Junya Hasegawa
Postdoctoral researcher	Toshiyoshi Yamamoto
Project Assistant Professor	Emi Tokuda, Shin Morioka
JSPS Research Fellow	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.

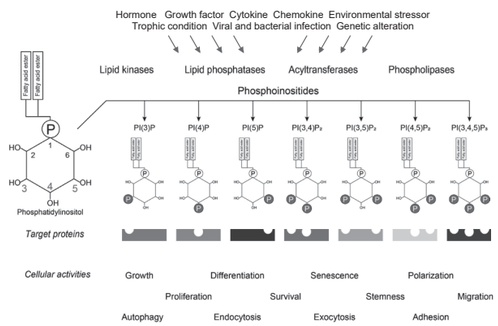


Fig.1 Phosphoinositide

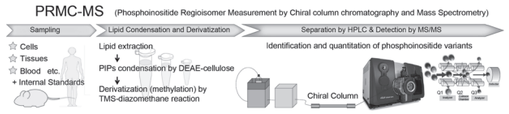


Fig.2 A new method for comprehensive analysis of phosphoinositide

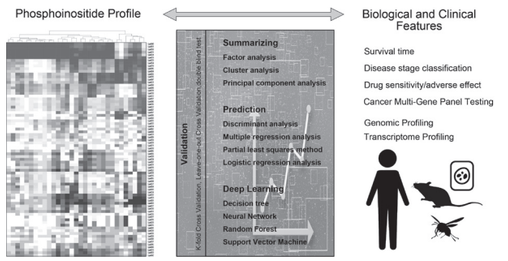


Fig.3 Phosphoinositide variants as molecular signatures for various diseases

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 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 and 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

1. Chessa TAM et al.: PLEKHS1 drives PI3Ks and remodels pathway homeostasis in PTEN-null prostate. Mol Cell 83, 2991-3009, 2023
2. Tran DM et al.: Attenuated cerebellar phenotypes in Inpp4a truncation mutants with preserved phosphatase activity. Dis Model Mech 16(7): dmm050169, 2023
3. Kiyoki Y et al.: The fatty acid elongase Elovl6 is crucial for hematopoietic stem cell engraftment and leukemia propagation. Leukemia 37, 910-913, 2023
4. Iguchi A et al.: INPP5D Modulates TREM2 Loss-of-Function Phenotypes in a Mouse Model of Alzheimer Disease. iScience 26, 106375, 2023
5. Miyake T et al.: Minimal upstream open reading frame of Per2 mediates phase fitness of the circadian clock to day/night physiological body temperature rhythm. Cell Rep. 42,112157, 2023
6. Arihisa W et al.: Lipid-correlated alterations in the transcriptome are enriched in several specific pathways in the postmortem prefrontal cortex of Japanese patients with schizophrenia. Neuropsychopharmacol Rep. 43, 403-413, 2023

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 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

In early embryogenesis, the primitive streak (PrS) generates the mesendoderm and is essential for organogenesis. However, because the PrS is a minute and transient tissue, elucidating the mechanism of its formation has been challenging. We performed comprehensive screening of two knockout mouse databases based on the fact that failure of PrS formation is lethal. We identified 812 genes involved in various cellular functions and responses that might be linked to PrS formation, with the category of greatest abundance being "Metabolism". In this study, we focused on genes of sphingolipid metabolism and investigated their roles in PrS formation using an *in vitro* mouse ES cell differentiation system. We show here that elevated intracellular ceramide negatively regulates gene expression essential for PrS formation and instead induces neurogenesis. In addition, sphingosine-1-phosphate (a ceramide derivative) positively regulates neural maturation. Our results indicate that ceramide regulates both PrS formation and the induction of neural differentiation (Figure 1).

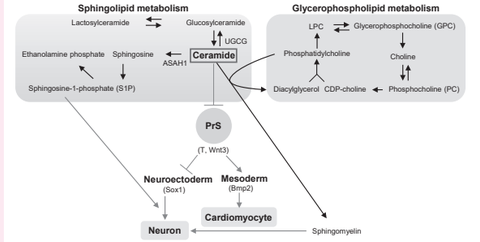


Fig.1.A schematic model of mouse primitive streak formation by ceramide metabolism

Publications

1. Jing Pu, Satoshi Kofuji, Yoshimi Okamoto-Uchida, Keiko Danzaki, Ruoxing Yu, Akira Suzuki, Satoshi Kitajima and Hiroshi Nishina (2023) Lethal phenotype-based database screening identifies ceramide as a negative regulator of primitive streak formation. ***Stem Cells*** 41(12):1142-1156. doi: 10.1093/stmcls/sxad071.

2. Noriyuki Azuma, Tadashi Yokoi, Taku Tanaka, Emiko Matsuzaka, Yuki Saida, Sachiko Nishina, Miho Terao, Shuji Takada, Maki Fukami, Kohji Okamura, Kayoko Maehara, Tokiwa Yamasaki, Jun Hirayama, Hiroshi Nishina, Hiroshi Handa, and Yuki Yamaguchi (2023) Integrator complex subunit 15 controls mRNA splicing and is critical for eye development. ***Human Molecular Genetics*** 32(12):2032-2045. doi: 10.1093/hmg/ddad034.

3. Hajime Tajima Sakurai, Hidefumi Iwashita, Satoko Arakawa, Alifu Yikelamu, Mizuki Kusaba, Satoshi Kofuji, Hiroshi Nishina, Munetaka Ishiyama, Yuichiro Ueno, and Shigeomi Shimizu (2023) Development of small fluorescent probes for the analysis of autophagy kinetics. ***iScience*** 26(7): 107218.

Department of Molecular Cell Biology

Professor Hiroshi Shibuya
Associate Professor Toshiyasu Goto
Assistant Professor Masahiro Shimizu

Overview

With no lysine kinase 1 (WNK1) phosphorylates and activates STE20/SPS1-related proline-alanine-rich protein kinase (SPAK) and oxidative stress responsive kinase 1 (OSR1) to regulate ion homeostasis in the kidney. Mutations in WNK1 result in dysregulation of the WNK1-SPAK/OSR1 pathway and cause pseudohypoaldosteronism type II (PHAII), a form of hypertension. WNK1 is also involved in the autosomal recessive neuropathy, hereditary sensory and autonomic neuropathy type II (HSANII). Mutations in a neural-specific splice variant of WNK1 (HSN2) cause HSANII. However, the mechanisms underlying HSN2 regulation in neurons and effects of HSN2 mutants remain unclear.

Introduction

A neuron-specific WNK1 splice variant, called HSN2, is expressed in the brain, dorsal root ganglia and sciatic nerve. Mutations in a neural specific exon of HSN2 cause the hereditary neuropathy called HSANII. HSANII is an autosomal recessive neuropathy characterized by sensory dysfunction, such as loss of pain, touch and temperature sensation, and limb abnormalities such as finger deformity and Charcot's joint. Although HSN2 is predicted to have an essential function in neurons, the precise mechanism by which HSN2 mutants cause HSANII is still unclear. To solve the problem, we focus on the function of HSN2 mutants in neural development.

1. HSN2 mutants suppress WNK signaling pathway by inhibiting the binding to wild-type WNK1, HSN2 and SPAK/OSR1.

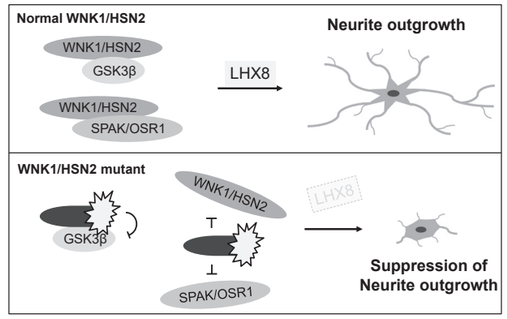
It has been reported that there are approximately 20 different mutations in HSN2 gene of all HSANII patients. Most mutations in HSN2 occur in the neural-specific HSN2 exon and cause deletion of subsequent domains. Previous report has shown that the C-terminal domain of WNK isoforms is important for interaction and auto-phosphorylation of WNK kinases. These data suggest that HSN2 mutants might not interact with and activate WNK kinases including WNK1 and WNK4 and downstream effectors SPAK and OSR1. To verify the hypothesis, we examined the binding of HSN2 mutants to WNK1, WNK4, HSN2, SPAK and OSR1. As a result, we found that HSN2 mutants could not interact with these proteins, suggesting that HSN2 mutants are unable to promote activation of downstream signalling.

To analyse whether HSN2 regulates activation of SPAK and OSR1, we checked phosphorylation of SPAK and OSR1 in mouse neuroblastoma Neuro2A cells treated with nerve growth factor (NGF)-containing serum-free medium. The phosphorylation of SPAK and OSR1 was observed at 10 to 15 minutes after treatment with NGF-containing medium. We next analysed the effect of HSN2 constructs on the phosphorylation of SPAK and OSR1 mediated by NGF, and found that HSN2 expression increased the level of SPAK and OSR1 phosphorylation identically to that following WNK1 expression. By contrast, the expression of HSN2 mutants suppressed the NGF-induced phosphorylation of SPAK and OSR1. These results indicate that HSN2 mutants cannot activate SPAK and OSR1 by inhibiting the binding to the effectors of WNK signalling pathway.

2. HSN2 mutants suppress neurite outgrowth and neural development.

We have previously reported that WNK1 is involved in induction of neural marker genes and neurite outgrowth in Neuro2A cells (Sato A *et al.* PLoS One, 2013). HSN2 may, therefore, have similar functions in neurite outgrowth and HSN2 mutants may exhibit loss of function. To examine whether HSN2 is involved in neurite outgrowth, we transiently expressed HSN2 in Neuro2A cells and observed that HSN2 induced neurite outgrowth identically to WNK1. We then examined the effect of HSN2 mutants on neurite outgrowth and found that HSN2 mutants could not induce neurite outgrowth. Next, we checked the effects of wild-type and mutant HSN2 on neurite outgrowth induced by NGF. NGF treatment induced neurite outgrowth, and the expression of WNK1 or HSN2 enhanced this induction. In contrast, the expression of HSN2 mutants suppressed neurite outgrowth in response to NGF-containing medium. We also examined the effect of WNK1, HSN2 and HSN2 mutants on neurite outgrowth of mouse primary cortical neuron. Consistent with the results from Neuro2A, neurite outgrowth of primary neuron was enhanced by WNK1 and HSN2 expression, but suppressed by HSN2 mutants.

We next analysed the expression of neural marker genes, Lhx8 and Choline acetyltransferase (ChAT) for cholinergic neurons and Glutamine acid decarboxylase 1 (Gad1) for GABAergic neurons. qPCR analysis indicated that NGF treatment greatly induced the expression of *Lhx8*, *ChAT* and *Gad1*, and that exogenous expression of WNK1 or HSN2 induced *Lhx8* and *ChAT* expression. WNK1 and HSN2 enhanced NGF-mediated expression of *Lhx8* and *ChAT*, but reduced that of *Gad1*. In contrast, expression of HSN2 mutants decreased *Lhx8* and *ChAT* expression and increased *Gad1* expression. These results indicate that WNK1 and HSN2 are involved in neurite outgrowth, but that HSN2 mutants fail in this activity.



Inhibitory effect of neurite outgrowth by WNK1/HSN2 mutant

3. HSN2 mutants have dominant negative functions for GSK3β.

Previously, we have also reported GSK3β to be a positive downstream regulator in the WNK-SPAK/OSR1 pathway, which regulates neurite outgrowth (Sato A *et al.* PLoS One, 2018). To identify the function of HSN2 mutants on GSK3β, we analyzed the interaction between GSK3β and HSN2 mutants. Immunoprecipitation analysis showed that HSN2 interacted more strongly with GSK3β than with WNK1. Interestingly, HSN2 mutants associated more robustly with GSK3β than with wild-type HSN2. From these results, we predicted that HSN2 mutants might suppress the binding of WNK1 and HSN2 to GSK3β by covering the binding sites of GSK3β. To confirm this hypothesis, we checked interaction of WNK1, HSN2 and GSK3β with HSN2 mutant expression. As a result, the binding of WNK1 and HSN2 to GSK3β was inhibited by expression of HSN2 mutants. Moreover, these mutants also prevented the homo-dimerization of WNK1 and HSN2 respectively, indicating that HSN2 mutants suppress the function of GSK3β in the WNK1/HSN2 pathway by preventing the interaction. We then examined the effect of HSN2 mutants on GSK3β function in neurite outgrowth. While transient GSK3β expression in Neuro2A cells enhanced neurite outgrowth and expression of cholinergic neural marker genes *Lhx8* and *ChAT*, the expression of HSN2 mutants suppressed these effects of GSK3β. Moreover, similar results were observed in mouse primary cortical neuron. These results suggest that HSN2 mutants preferentially bind to GSK3β and function as dominant negative mutants which suppress the function of GSK3β in neurite outgrowth.

In conclusion, we showed the biochemical function of HSN2 mutants in neural development, providing insight into the pathological mechanism of HSANII.

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1. Goto, T., Michiue, T. and Shibuya, H. (2023). *ccl19* and *ccl21* affect cell movements and differentiation in early *Xenopus* development. **Dev. Growth Differ.** 65, 175-189.
2. Nishita, M., Kamizaki, K., Hoshi, K., Aruga, K., Nishikaku, I., Shibuya, H., Matsumoto, K. and Minami, Y. (2023). Rho family small GTPase Rif regulates Wnt5a-Ror1-Dvl2 signaling and promotes lung adenocarcinoma progression. **J. Biol. Chem.** 299, 105248.

Department of Stem Cell Regulation

Professor	Tetsuya Taga
Junior Associate Professor	Kouichi Tabu
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Technical Assistant	Marika Nodera
Technical Assistant	Toru Ishikawa (August 2023-)

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. Development of niche-mimicking polymers for neural stem cell maintenance

Neural stem cells (NSCs) are the common precursor cells that produce neurons and glial cells, and they are crucial for the development and maintenance of homeostasis in the central nervous system from the embryonic stage through to old age. Therefore, elucidating the molecular basis underlying the maintenance of NSC self-renewal is a critical foundation for understanding the pathogenesis and developing therapies for intractable diseases caused by abnormalities in the regulation of NSCs.

In this study, we approached this challenge through an unprecedented strategy by developing a synthetic polymer that mimics the neural stem cell niche. Through collaboration with Professor Mark Bradley of the Queen Mary University of London, we have successfully identified a novel synthetic polymer, PA518 [MEMA : DEAEMA :St=40:30:30], that can maintain mouse NSCs in a culture medium without serum and growth factors, which are traditionally conditions for NSC maintenance.

To elucidate the molecular basis underlying the maintenance of NSCs by PA518, it is essential to scale up the NSC culture. By adjusting the PA518 monomer content, we improved the polymer synthesis method, enhancing the adhesion properties between the NSCs and the polymer at specific monomer ratios. Furthermore, embryonic mouse NSCs cultured on PA518, even in an FGF2- and serum-free differentiation-inducing culture system, strongly retained the expression of nestin, an undifferentiated NSC marker. Thus, we found that the monomer content during polymer synthesis significantly affects NSC adhesion and the maintenance of their undifferentiated state. This study has provided tools for identifying the signaling pathways underlying the NSC maintenance by PA518. Additionally, by refining the polymer synthesis method, we have opened up promising avenues for enhancing niche functions and potentially developing efficient NSC amplification technologies (Figure 1).

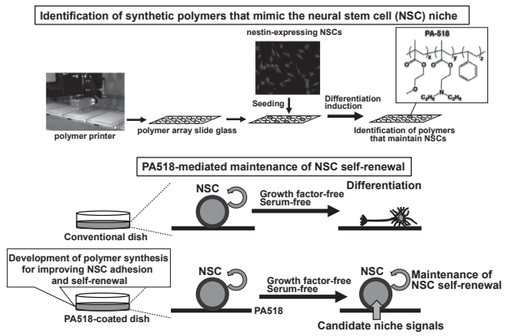


Figure 1. Identification of a niche mimicking synthetic polymer scaffold that maintains neural stem cells (see the text for the polymer structure)

2. Identification of a novel gene, Rasip1, that contributes to the proliferation and maintenance of hematopoietic stem cells at the embryonic stage

Hematopoietic stem cells, which give rise to all blood cells, first appear in small aggregates of blood cells (hematopoietic cell clusters) that form in the lumen of the aorta in midgestation embryos in mammals, we have previously reported that overexpression of the transcription factor Sox17 into fetal hematopoietic stem cells derived from hematopoietic cell clusters, which are difficult to be maintained in vitro, led to maintenance of stem cell activity on culture dishes, but the mechanism has not yet been elucidated. In this study, we have shown that Sox17 induces expression of the Ras interacting protein 1 (Rasip1) gene by directly acting on its regulatory promoter region. Overexpression of Rasip1 in hematopoietic cluster-forming cells led to the maintenance of clusters with hematopoietic activity, 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 specified cells. Based on the findings of our study, a new technology to increase the number of hematopoietic stem cells from bone marrow in vitro is expected to be developed (Figure 2).

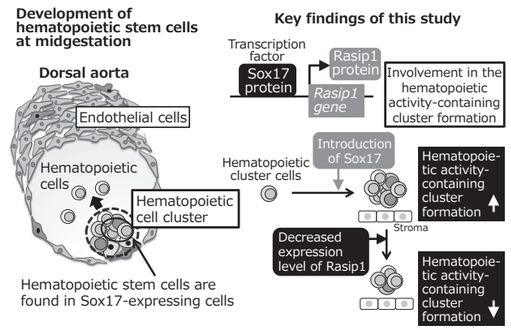


Figure 2. Rasip1 is involved in the formation of hematopoietic stem cell-containing hematopoietic cell clusters in midgestation mouse embryo.

3. Niche-mimicking polymer hydrogel-based identification of molecular targets for pancreatic cancer 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 relapse and are considered as a promising target to eradicate cancers. In light of the evidence that cancer stem cells (CSCs) are maintained in the specialized microenvironment called niche, exploring its components provides a clue for developing effective strategies against malignant cancers. However, CSC niche is composed of biologically and physiochemically complicated factors. Thus, a multidisciplinary approach that comprehensively but more precisely recapitulates their complex interactions has been considered necessary for the identification of promising targets. In this year we applied hundreds of polymers and their hydrogels as niche-mimicking research materials to elucidate niche characteristics (Figure 3). Firstly, polymer microarray screening identified PA531 as a functional polymer that specifically expands pancreatic CSCs. Its biological activity was further optimized by hydrogelation under different monomer concentrations and finally PA531-HG4 was developed as a best material to expand pancreatic CSCs. Proteome analysis identified fetuin-B and Angiotensinogen specifically bound with PA531-HG4, and their gene expression is found to be significantly associated with the worse and better outcome in pancreatic cancer patients, respectively. As fetuin-B is expressed mainly in the liver, it might be involved in CSC maintenance not only in primary pancreatic tumors but also in liver metastasis.

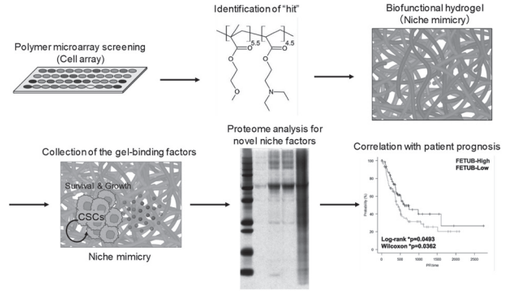


Figure 3. Niche-mimicking polymer hydrogel-based exploration of extracellular targets in cancer

Publications

[Original Article]

Kashiwagi T, Takazawa Y, Kagawa T, Taga T. Organization of self-advantageous niche by neural stem/progenitor cells during development via autocrine VEGF-A under hypoxia. Inflamm Regen 43(1):8, 2023

Melig G, Nobuhisa I, Saito K, Tsukahara R, Itabashi A, Kanai Y, Kanai-Azuma M, Osawa M, Oshima M, Iwama A, Taga T. A Sox17 downstream gene Rasip1 is involved in the hematopoietic activity of intra-aortic hematopoietic clusters in the midgestation mouse embryo. Inflamm Regen 43(1):41, 2023

Murota Y, Nagane M, Wu M, Santra M, Venkateswaran S, Tanaka S, Bradley M, Taga T, Tabu K. A niche-mimicking polymer hydrogel-based approach to identify molecular targets for tackling human pancreatic cancer stem cells Inflamm Regen 43(1):46, 2023

Department of Homeostatic Medicine

Professor　Fumiko Toyoshima, Ph.D.

Each organ in the body undergoes changes in form and function as life stages progress. For example, during pregnancy, multiple organs of the mother are remodeled to prepare the maternal environment to support the growth of the fetus. Ageing and obesity induce pathological organ remodeling with chronic inflammation. Our laboratory aims to elucidate the mechanisms of physiological and pathological organ remodeling and to understand how the mechanisms contribute to maintain homeostasis in the body and the onset of disease. We are also developing regenerative medicine and anti-ageing technologies that utilize organ remodeling mechanisms in the body.

Research Projects

1. Physiological skin expansion during pregnancy

During pregnancy, the size of various maternal organs changes. This change in organ size is essential for maternal organ functions that support the acquisition of pregnancy-specific metabolic functions and foetal development, but the underlying mechanisms are unknown. In recent years, it has been shown that the proliferation and differentiation of various maternal tissue stem cells, such as neural stem cells, mammary stem cells and haematopoietic stem cells, is enhanced during pregnancy, highlighting the role of tissue stem cells in the regulation of maternal organ size.

Our laboratory has studied the mechanism of skin expansion in the maternal abdomen during pregnancy. We have found that in the abdominal skin of pregnant mice, highly proliferative cells from epidermal stem cells are emerged in the basal layer of the epidermis. This cell population expresses the transcription factor Tbx3, and abdominal epidermis-specific knockout of Tbx3 (Tbx3cKO) reduced the number of highly proliferative cells and markedly suppressed pregnancy-induced skin expasion. These Tbx3-positive basal cells are induced by secretory signals (e.g. Igfbp2) from α-SMA/vimentin-positive cells, which increase in the dermis during gestation, indicating the existence of a dermal-epidermal interaction mechanism for skin expansion. Furthermore, Tbx3-positive basal cells require skin vasculature to maintain their stemness. These cells were found to emerge during gestation, dependent on the increase in skin blood vessels, and to differentiate and be expelled from the epidermis after parturition with vascular regression. In addition, tension loading of the skin also increased skin blood vessels in non-pregnant mice and induced Tbx3-positive basal cells, indicating that blood vessels and tension are triggers for skin expansion (Figure1).

Currently, we aim to elucidate the mechanisms by which blood vessels and tissue force fields induce skin remodeling. In addition, the development of skin regenerative medicine technology using this mechanism is underway.

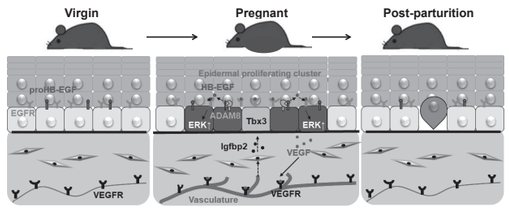


Figure1. Abdominal skin remodeling during pregnancy

2. Vascular atrophy causes dermal stiffness and epidermal stem cell aging

Proliferation and differentiation of epidermal stem cells is essential for skin homeostasis. Epidermal stem cells deteriorate their stemness with age, their adhesion to the basement membrane becomes weaker and the direction of cell division becomes abnormal. It has been reported that the age-related dysfunction of epidermal stem cells is due to internal cellular changes such as DNA damage induced by oxidative stress. However, the changes in the environment surrounding epidermal stem cells that occur with age remain unresolved.

Our group showed that the dermis is markedly stiffened in the plantar skin of ageing mice, which causes persistent activation of the mechanosensor Piezo1 in epidermal stem cell, leading to excessive calcium influx into the cells and inducing epidermal stem cell dysfunction. It has also been shown that age-related stiffening of the dermis is caused by degeneration of skin vasculature, and that artificially inducing skin blood vessels suppresses age-related hardening of the dermis and the functional decline of epidermal stem cells. Furthermore, Pentraxin 3 (Ptx3), which is secreted by fibroblasts in ageing skin, is one of the factors responsible for the regression of skin blood vessels, and Ptx3 KO mice show reduced age-related dermal stiffening and epidermal stem cell dysfunction. These results reveal a novel mechanism of skin ageing, in which the expression of Ptx3 is increased from dermal fibroblasts with age, which induces vascular atrophy and dermal stiffening, and triggers age-related alterations in epidermal stem cells due to persistent calcium influx via Piezo1(Figure2).

In humans, Ptx3 accumulated more in the skin of older people than younger people, suggesting that Ptx3 may be one of the causes of skin ageing in humans. The development of anti-aging technologies using Ptx3 as a seed is expected.

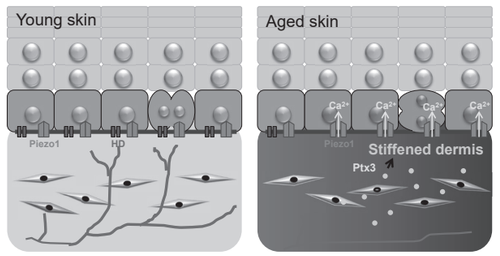


Figure2. Vasculature atrophy causes dermal stiffening and epidermal stem cell aging

3. Maternal liver remodeling regulates gestational diabetes

The maternal liver markedly enlarged during pregnancy, but the mechanism and the relationship to maternal metabolism and foetal development are unknown. We found that hepatocyte proliferation is spatiotemporally regulated during gestation. In mice, the proliferation of hepatocytes in the portal vein region transiently increased during mid-pregnancy (gestation day 8), whereas the proliferation of hepatocytes in the central venous region increased during late gestation (gestation day 16). Suppression of hepatocyte proliferation from mid-pregnancy using AAV8-p21 induced gestational diabetes-like symptoms, such as increased maternal blood glucose levels, impaired glucose tolerance and fetal overgrowth in late pregnancy. In the liver of these mice, the expression of genes related to glucose metabolism was altered, with a decrease in liver glycogen levels and a corresponding increase in placental glycogen levels. These symptoms were not observed when hepatocyte proliferation was inhibited from day 12 of gestation, suggesting that hepatocyte proliferation in mid-gestation governs maternal glucose metabolism in late gestation. In addition, we identified Hmmr, a cofactor for the hyaluronan receptor, as a key regulator for hepatocyte proliferation in the portal region during mid-pregnancy, and knockdown of Hmmr in hepatocytes resulted in gestational diabetes-like symptoms. These results demonstrate that Hmmr-mediated proliferation of hepatocytes in the portal vein region in mid-pregnancy is important for the regulation of maternal glucose metabolism by increasing the glycogen storage capacity of the maternal liver.

Elevated blood glucose levels due to abnormal glucose metabolism during pregnancy can also cause hyperglycaemia and hyperinsulinaemia in the fetus, inducing developmental abnormalities and complications. Although insulin-suppressing hormones secreted from the placenta and insulinolysis are well known to regulate glucose metabolism during pregnancy, no studies have focused on maternal liver remodeling. Elucidating the biological systems that induce gestational liver remodeling is expected to lead to the development of new preventive strategies against gestational diabetes mellitus.

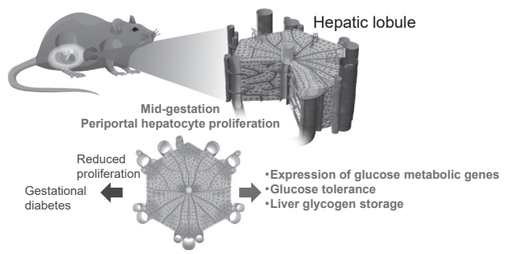


Figure3. Periportal hepatocyte proliferation at mid-gestation prevents gestational diabetes

Publications

Satoshi Kozuki, Mio Kabata, Satoko Sakurai, Keiko Iwaisako, Tomomi Nishimura, Masakazu Toi, Takuya Yamamoto, Fumiko Toyoshima. Periportal hepatocyte proliferation at midgestation governs maternal glucose homeostasis in mice. ***Commun. Biol.*** 6, 1226, 2023

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】

- TBK1 kinase is required for the clearance of damaged mitochondria, but the molecular mechanisms remained obscure. We discovered that Optineurin (OPTN) forms contact sites between damaged mitochondria and isolation membranes to activate TBK1. Additionally, we found that artificial antibodies inhibiting the interaction between OPTN and TBK1 suppress TBK1 activation and the subsequent degradation 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 are working to elucidate the mechanisms by which resistance to chemotherapeutic agents is acquired.

【Neuropathology】

- Discovery of a new propagation pathway of neurodegenerative disease proteins via brain lymphatic system.
- Development of a new therapeutics method of Charcot Marie Tooth disease type 1A based on genome editing.

【Molecular Neuroscience】

- Glial modulation of the parallel memory formation
- Generation of Prokineticin2 (Prok2)^{rTA} knock-in mice
- Astrotactin2 knockout mice show some emotional or cognitive impairments

【Pathological Cell Biology】

- We found that casein kinase 1ε/δ is involved in the pathogenesis of familial Parkinson's disease caused by CHCHD2 gene mutations.
- We have developed a novel method to visualize GOMED, a new proteolytic degradation mechanism.

【Neuroinflammation and Repair】

- Metabolites of di-homo-gamma-linolenic acid are identified as the initiator of neural repair after brain injury.
- We have clarified the molecular and cellular mechanisms which diminish spontaneous brain repair after brain injury and have developed the drug that sustains brain functional recovery.

Department of Biomolecular Pathogenesis

Professor	Noriyuki Matsuda
Associate Professor	Koji Yamano
Assistant Professor	Fumika (Komatsuya-)Koyano
Postdoctoral Researcher	Waka Kojima, Aiko Watanabe

Background

Various organelles exist in cells, but some of them are damaged in the process of performing their functions and then become damaged ones. To maintain organelle function in the cell, such damaged organelles must be removed by selective autophagy. During this process, only damaged organelles must be recognized, identified, and degraded. Recent advances in our understanding of the molecular mechanisms underlying selective organelle degradation and physiological significance of this process have revealed the importance of ubiquitin and autophagy adapters. Moreover, their relevance to neurodegenerative diseases have also been revealed. We have studied the mechanism by which PINK1 and Parkin, the causative gene products of hereditary recessive Parkinson's disease (PD), label damaged mitochondria with ubiquitin and lead to subsequent autophagic degradation (mitophagy). We intend to elucidate the molecular mechanisms of selective organelle degradation and to understand its pathophysiological significance.

Research Projects

1. OPTN provides the specialized niche for phosphorylation and activation of TBK1 which is required for mitophagy:

OPTN (Optineurin) is an autophagy adaptor protein with ubiquitin-binding activity that promotes autophagic degradation of its targets by bridging autophagy-related factors and ubiquitin added to the targets. We found that OPTN interacts with the autophagy essential factor ATG9 and that this process is important for Parkin-dependent mitophagy (Yamano, *J. Cell Biol.*, 2020). However, the role of OPTN in mitophagy is not fully understood and requires further analyses.

TBK1 (Tank-binding kinase 1) is a Ser/Thr kinase involved in various cellular processes. It has been reported that TBK1 is involved in the phosphorylation of autophagy adaptors in mitophagy (e.g., Heo et al., *Mol Cell* 2015), but how TBK1 is activated during mitophagy remains obscure.

We found that autophosphorylation (pS172) of TBK1 that is required for its activation transiently increases during mitophagy induction for about 30 min and then rapidly decreases within 2-3 h. This "decrease in TBK1 autophosphorylation" is suppressed by lysosome inhibitors. Knockout (KO) of OPTN dramatically reduced phosphorylated TBK1 (TBK1pS172) levels, while KO of NDP52, another autophagy adaptor similar to OPTN, had little effect on TBK1pS172. These results indicate that activated TBK1 is rapidly degraded in lysosomes via the autophagy pathway and that OPTN is the rate-limiting factor for mitophagy-induced TBK1 phosphorylation and activation.

Next, we found that OPTN accumulates at contact sites between damaged mitochondria and isolation membranes (hereafter referred to as Mt-AP CS) following mitophagy induction. This finding suggests at the level of subcellular localization that OPTN bridges the gap between ubiquitin on damaged mitochondria and autophagy-related factors on the isolation membrane. Interestingly, Mt-AP CS localization of OPTN was essential for TBK1 activation. OPTN mutations such as 4LA and F178A, which changed its localization from Mt-AP CS to the whole mitochondria, markedly inhibited mitophagy-induced TBK1 activation. Similarly, disruption of genes required for isolation-membrane formation that caused loss of Mt-AP CS itself also inhibited mitophagy-induced TBK1 activation. These results indicate that for full activation of TBK1, it is not sufficient for OPTN to associate with mitochondria, but it must be localized at Mt-AP CS. Furthermore, after the process of examining whether OPTN or TBK1 functions as an upstream factor, we found that both form an interdependent positive-feedback loop. These results provide insight into how OPTN and TBK1 initiate autophagosome formation on the surface of damaged mitochondria through complicated interactions (see schematic diagram in the Summary Figure "Highlight").

This study is under revision in EMBO Journal at the end of 2023.

2. Other research topics:

In addition to the above-mentioned studies, we are also studying on the following topics related to selective organelle degradation or Parkinson's disease.

(1) Isolation and functional analysis of a novel autophagy regulator: We have isolated the BCAS3-C16Orf17 complex as a novel factor that is rapidly recruited onto the siolation membrane near damaged mitochondria following mitophagy induction (Kojima et al., Autophagy 2021). It is becoming clear that this complex is a novel autophagy-related factor. Currently, we are studying its molecular function and physiological significance.

(2) Functional analysis of DJ-1, a causative factor of hereditary PD: DJ-1 is a causative gene product of hereditary recessive PD, but its molecular function is still controversial among researchers due to various hypotheses. We have focused on the oxaldehyde detoxification activity of DJ-1 to uncover its genuine molecular function.

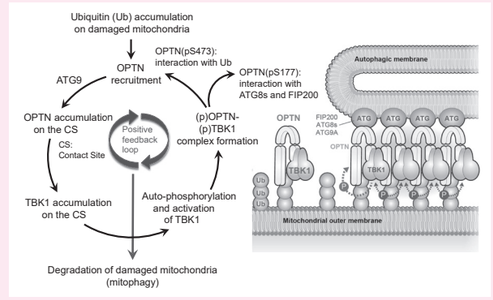
(3) Analysis of Pexophagy: We have found that ubiquitylation is also important in peroxisome-specific autophagy called Pexophagy. We are thus analyzing the molecular mechanisms how ubiquitylation is converted into a signal for Pexophagy.

We intend to continue our research to understand the molecular mechanisms underlying selective organelle degradation and to elucidate the mechanisms of disease pathogenesis focusing on hereditary PD.

Highlight

Mechanisms of OPTN-dependent TBK1 activation upon mitophagy induction.

PINK1 and Parkin cooperate to add short chains of ubiquitin (Ub) to the surface of damaged mitochondria, which are then recognized by OPTN possessing ubiquitin-binding activity. The previous hypothesis was that TBK1 phosphorylates OPTN and then OPTN synthesizes isolation membrane de novo in the vicinity of mitochondria via binding to autophagy-related factors (ATG). Our current study indicates that phosphorylation and activation of TBK1 require the formation of the isolation membrane (or the niche formed in the contact site of damaged mitochondria and the isolation membrane). Interaction between TBK1 and OPTN in this region is important for TBK1 activation and subsequent progress of mitophagy.



Publications

1. Akabane, S., Watanabe, K., Kosako, H., Yamashita, SI., Nishino, K., Kato, M., Sekine, S., Kanki, T., Matsuda, N., Endo, T., and Oka, T. TIM23 facilitates PINK1 activation by safeguarding against OMA1-mediated degradation in damaged mitochondria. ***Cell Rep.*** 42, 112454 (2023).

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

Department of Biodefense Research

Professor	Toshiaki Ohteki, DDS, Ph.D.
Associate Professor	Taku Sato, Ph.D. (~Sep 30, 2023), Masashi Kanayama, Ph.D. (Oct 1, 2023~)
Assistant Professor	Masashi Kanayama, Ph.D. (~Sep 30, 2023)
Adjunct Lecturer	Nobuyuki Onai, Ph.D., Yasuhiro Murakawa, MD, Ph.D.
Research Technician	Kisho Shiseki (~Oct 31, 2023), Toyoki Hayashi (~March 31, 2023)
Research Technician	Takako Akashi (April 1, 2023~)
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. Based on 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). In addition, although Common Lymphoid Progenitor (CLP) cell-derived pDCs are numerically superior to CDP-derived pDCs, we, as an international joint proposal, reported that CLP-derived pDCs should rather be classified as innate lymphocytes because they lack antigen-presenting capacity and do not meet the definition of DCs (***Nat Rev Immunol*** 2023) (**Figure 1**).

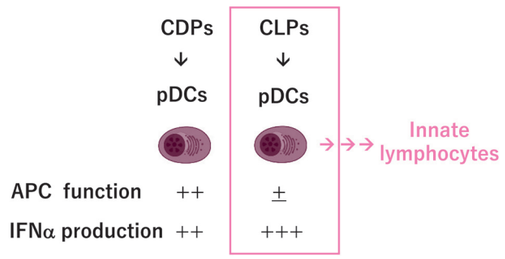


Fig.1 Reclassifying pDCs as innate lymphocytes
Ziegler-Heitbrock L, Ohteki T, Ginhoux F et al., *Nat Rev Immunol 2023*

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) 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*** 2023) enhances the supply of myeloid system cells (**Figure 2**).

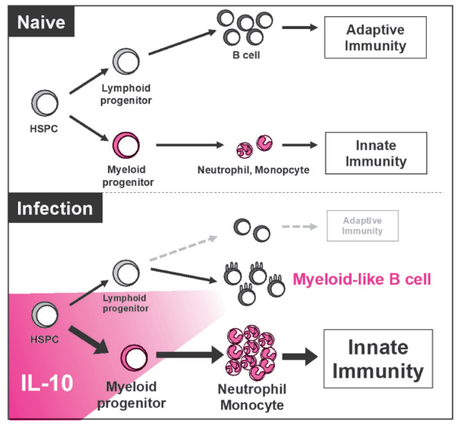


Fig.2 Increased production of innate immune cells by a new B cell subset that emerges during infection
Kanayama M et al., *J Exp Med 2023*, cited from Graphic Abstract with modifications

2. Research on tissue stem cells

1) Tissue homeostasis and its breakdown based on 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).

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

2) Establishment of human tongue cancer organoid biobank

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 multi-center 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 (submitted).

Publications

1. Abe S, Asahi T, Hara T, Cui G, Shimba A, Tani-Ichi S, Yamada K, Miyazaki K, Miyachi H, Kitano S, Nakamura N, Kikuta J, Vandenbon A, MiyazakiM, Yamada R, Ohteki T, Ishii M, Sexl V, Nagasawa T, and Ikuta K. Hematopoietic cell-derived IL-15 supports NK cell development in scattered and clustered localization within the bone marrow. ***Cell Rep*** 42: 113127 (2023).
2. 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*** 220:e20221221 (2023).
3. Ziegler-Heitbrock L, Ohteki T, Ginhoux F, Shortman K, and Spits H. Reclassifying plasmacytoid dendritic cells as innate lymphocytes. ***Nat Rev Immunol*** 23:1-2 (2023).

Personnel Changes

Moving in:
Yuki Yamada (master's course), Joh Tohnai (doctoral course, Jichi Medical University), Takako Akashi (technical assistant), and Shun Ishikawa (graduate research student) joined us.

Moving out:
Taku Sato was elected as a professor at Nippon Medical School, School of Medicine (Biochemistry and Molecular Biology).
Yuta Izumi finished the doctoral course, Eriko Ohashi and Mai Nakagawa completed the master's course, and Kisho Shiseki and Toyoki Hayashi (technical assistants) completed their term of office.

Department of Neuropathology

Professor	Hitoshi Okazawa
Project Lecturer/Adjunct lecturer	Haruhisa Inoue, Masaki Sone, Kyota Fujita
Project Associated professor	Hidenori Homma
Lecturer	Hikari Tanaka
Assistant professor	Maiko Inotsume

[Outline]

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

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

[This year's progress]

1. A secret passage for mutant protein to invade the brain

In a study published recently in *Cell Reports*, we have shown that a mutated version of a protein called α-synuclein propagates to various cerebral regions through the lymphatic system and then aggregates. In neurodegenerative diseases including Parkinson's disease, disease proteins are considered to be transmitted to remote brain regions in the aggregate form via synapse, while the experiments were conducted by using preformed fibrils, which excluded the analysis of other forms such as oligomer or monomer α-synuclein aggregates.

To further investigate how α-synuclein move around in the brain, we injected small amounts of viral particles that express monomer mutant α-synuclein.

Interestingly, fluorescent α-synuclein was detected in remote regions two weeks after injection, indicating an early spreading of mutant α-synuclein in the brain and the propagated proteins were in the monomer form.

We followed the three-dimensional distribution of α-synuclein in the brain and found fluorescent α-synuclein in the glymphatic system. The glymphatic system is involved in draining and renewing fluid from the brain and eliminating toxins, but it could also distribute toxic substances throughout the brain. We also observed the presence of fluorescent α-synuclein in the matrix surrounding neurons and in the cytosol of neurons. This finding suggested that fluorescent α-synuclein was taken up by the extracellular matrix and, subsequently, by neurons.

This study shows how monomeric α-synuclein propagates through the glymphatic system in a different way from the fibrils Thus, targeting these early events, α-synuclein monomer and brain lymphatic system, may limit the progression of Parkinson's disease.

2. Antagonistic roles of canonical and Alternative-RPA in disease-associated tandem CAG repeat instability

Seventy neurodegenerative diseases are caused by expansions of gene-specific tandem repeat sequences, several of which are associated with (CAG),(CTG) expansions, including Huntington disease (HD) and multiple spinocerebellar ataxias (SCAs). Inherited expansions continue to somatically expand with age in affected tissues, suggesting that ongoing expansions drive disease age of onset (AOO), progression, and severity.

Our data show Alt-RPA enhances in vitro slipped-CAG repair, while pA1 blocks slipped-DNA repair. These results indicate that RPA/Alt-RPA modulate CAG repeat instability and support our previous result that RpA1 could be used for gene therapy of SCA1.

3. Promising new treatment for a common hereditary nerve disease

In a study published last month in Communications Medicine, we have unveiled a groundbreaking genome-editing technique. This innovation holds promise for treating Charcot–Marie–Tooth (CMT), a relatively common hereditary nerve disease that affects the nerves and currently has no clinical treatments.

CMT is characterized by altered sensation and muscle weakness in the limbs and affects 10 to 80 people per 100,000. The most common CMT subtype is known as CMT1A and is caused by a duplication of the gene encoding peripheral myelin protein 22 (PMP22), leading to high levels of this protein in affected individuals.

We created an iPS cell model by taking cells from a patient with CMT1A and differentiated the iPS cells to Schwann cells. We then used an original genome-editing technique integrated to AAV vectors, to delete the unnecessary genome copy and to decrease the amount of PMP22 protein.

Because both higher and lower PMP22 levels can lead to different types of nerve diseases (known as neuropathies), we need to be very careful about how much we reduced PMP22. However, our genome editing resolve this issue. We also confirmed that 20-40% of infected Schwann cells were genome-edited to delete the unnecessary copy.

Publications

1. Yoshioka Y, Taniguchi JB, Homma H, Tamura T, Fujita K, Inotsume M, Tagawa K, Misawa K, Matsumoto N, Nakagawa M, Inoue H, *Tanaka H, *Okazawa H. AAV-mediated editing of PMP22 rescues Charcot-Marie-Tooth disease type 1A features in patient-derived iPS Schwann cells. *Commun Med (Lond)*, November 28, 2023, 3(1):170. doi: 10.1038/s43856-023-00400-y.
2. Gall-Duncan T, Luo J, Jurkovic CM, Fischer LA, Fujita K, Deshmukh AL, Harding RJ, Tran S, Mehkary M, Li V, Leib DE, Chen R, Tanaka H, Mason AG, Lévesque D, Khan M, Razzaghi M, Prasolava T, Lanni S, Sato N, Caron MC, Panigrahi GB, Wang P, Lau R, Castel AL, Masson JY, Tippett L, Turner C, Spies M, La Spada AR, Campos EI, Curtis MA, Boisvert FM, Faull RLM, Davidson BL, Nakamori M, Okazawa H, Wold MS, *Pearson CE. Antagonistic roles of canonical and Alternative-RPA in disease-associated tandem CAG repeat instability. *Cell*, October 26, 2023, 186(22):4898-4919.e25. doi: 10.1016/j.cell.2023.09.008.
3. Fujita K, Homma H, Jin M, Yoshioka Y, Jin X, Saito Y, Tanaka H, *Okazawa H. Mutant α-synuclein propagates via the lymphatic system of the brain in the monomeric state. *Cell Reports*, August 29, 2023, 42(8):112962. doi: 10.1016/j.celrep.2023.112962.
4. *Shiwaku H, Katayama S, Gao M, Kondo K, Nakano Y, Motokawa Y, Toyoda S, Yoshida F, Hori H, Kubota T, Ishikawa K, Kunugi H, Ikegaya Y, Okazawa H, Takahashi H. Analyzing schizophrenia-related phenotypes in mice caused by autoantibodies against NRXN1α in schizophrenia. *Brain Behav Immun*, July, 2023, 111:32-45. doi: 10.1016/j.bbi.2023.03.028.
5. Jin X, Tanaka H, Jin M, Fujita K, Homma H, Inotsume M, Yong H, Umeda K, Kodera N, Ando T, *Okazawa H. PQBP5/NOL10 maintains and anchors the nucleolus under physiological and osmotic stress conditions. *Nat Commun*, January 4, 2023, 14(1):9. doi: 10.1038/s41467-022-35602-w.

Department of Molecular Neuroscience

Professor Kohichi Tanaka
Assistant Professor Yuichi Hiraoka

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

1. Functions of glutamate transporters in the brain

Glutamate is a major excitatory neurotransmitter and plays an important role in neuronal plasticity and neurotoxicity in the central nervous system. Glutamate transport proteins provide the mechanism by which synaptically released glutamate is inactivated and kept below toxic levels in the extracellular space. By now, five subtypes of high-affinity glutamate transporters have been identified in the mammalian brain. Our lab studies the physiological and pathological roles of glutamate transporter subtypes using subtype-specific knockout mice (Fig. 1).

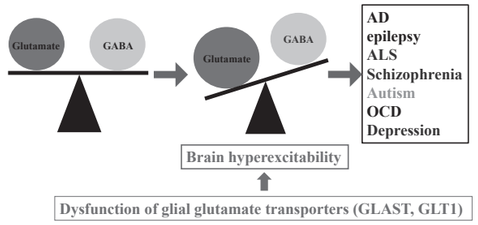


Fig.1 Glutamate transporter dysfunction leads to neuropsychiatric diseases

2. Glial modulation of the parallel memory formation

Actions from glial cells could affect the readiness and efficacy of learning and memory. Using a mouse cerebellar-dependent horizontal optokinetic response motor learning paradigm, short-term memory (STM) formation during the online training period and long-term memory (LTM) formation during the offline rest period were studied. A large variability of online and offline learning efficacies was found. The early bloomers with booming STM often had a suppressed LTM formation and late bloomers with no apparent acute training effect often exhibited boosted offline learning performance. Anion channels containing LRRC8A are known to release glutamate. Conditional knockout of LRRC8A specifically in astrocytes including cerebellar Bergmann glia resulted in a complete loss of STM formation while the LTM formation during the rest period remained. Optogenetic manipulation of glial activity by channelrhodopsin-2 or archaerhodopsin-T (ArchT) during the online training resulted in enhancement or suppression of STM formation, respectively. STM and LTM are likely to be triggered simultaneously during online training, but LTM is expressed later during the offline period. STM appears to be volatile and the achievement during the online training is not handed over to LTM. In addition, we found that glial ArchT photoactivation during the rest period resulted in the augmentation of LTM formation. These data suggest that STM formation and LTM formation are parallel separate processes. Strategies to weigh more on the STM or the LTM could depend on the actions of the glial cells.

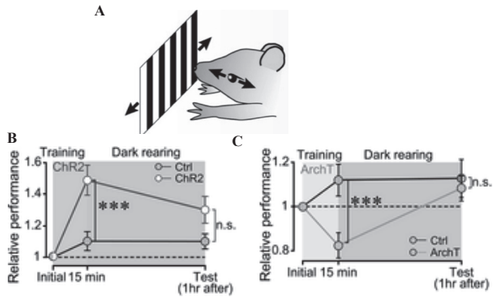


Fig.2 Glial optogenetic manipulation of online learning
A: Horizontal optokinetic response (HOKR) experiment
B: Relative performance at 15 min after training was significantly larger in the glial-ChR2-photoactivated mice but not at 1 h after rest.
C: Relative performance at 15 min after training was significantly decreased in the glial-ArchT-photoactivated mice but not at 1 h after rest.

3. Generation of Prokineticin2 (Prok2)^{tTA} knock-in mice

Prokineticin 2 (Prok2) is a small protein expressed in a subpopulation of neurons in the suprachiasmatic nucleus (SCN), the primary circadian pacemaker in mammals. Prok2 has been implicated as a candidate output molecule from the SCN to control multiple circadian rhythms. Genetic manipulation specific to Prok2-producing neurons would be a powerful approach to understanding their function. Here, we report the generation of Prok2-tTA knock-in mice expressing the tetracycline transactivator (tTA) specifically in Prok2 neurons and an application of these mice to in vivo recording of Ca^{2+} rhythms in these neurons. First, the specific and efficient expression of tTA in Prok2 neurons was verified by crossing the mice with EGFP reporter mice. Prok2-tTA mice were then used to express a fluorescent Ca^{2+} sensor protein to record the circadian Ca^{2+} rhythm in SCN Prok2 neurons in vivo. Ca^{2+} in these cells showed clear circadian rhythms in both light-dark and constant dark conditions, with their peaks around midday. Notably, the hours of high Ca^{2+} nearly coincided with the rest period of the behavioral rhythm. These observations fit well with the predicted function of Prok2 neurons as a candidate output pathway of the SCN by suppressing locomotor activity during both daytime and subjective daytime.

4. Astrotactin 2 (ASTN2) regulates emotional and cognitive functions

Astrotactin2 (ASTN2) regulates neuronal migration and synaptic strength through the trafficking and degradation of surface proteins. Deletion of ASTN2 in copy number variants has been identified in patients with schizophrenia, bipolar disorder, and autism spectrum disorder in copy number variant (CNV) analysis. Disruption of ASTN2 is a risk factor for these neurodevelopmental disorders, including schizophrenia, bipolar disorder, autism spectrum disorder, and attention deficit hyperactivity disorder. However, the importance of ASTN2 in physiological functions remains poorly understood. To elucidate the physiological functions of ASTN2, we investigated whether deficiency of ASTN2 affects cognitive and/or emotional behaviors and neurotransmissions using ASTN2-deficient mice. Astn2 knockout (KO) mice produced by CRISPR/Cas9 technique showed no obvious differences in physical characteristics and circadian rhythm. Astn2 KO mice showed increased exploratory activity in a novel environment, social behavior and impulsivity, or decreased despair-, anxiety-like behaviors and exploratory preference for the novel object. Some behavioral abnormalities, such as increased exploratory activity and impulsivity, or decreased exploratory preference were specifically attenuated by risperidone, but not by haloperidol. While, the both drugs did not affect any emotion-related behavioral abnormalities in Astn2 KO mice. Dopamine contents were decreased in the striatum, and serotonin or dopamine turnover were increased in the striatum, nucleus accumbens, and amygdala of Astn2 KO mice. In morphological analyses, thinning of neural cell layers in the hippocampus, reduction of neural cell bodies in the prefrontal cortex, and decrease in spine density and PSD95 protein in both tissues were observed in Astn2 KO mice. The present findings suggest that ASTN2 deficiency develops some emotional or cognitive impairments related to monoaminergic dysfunctions and abnormal neuronal morphogenesis with shrinkage of neuronal soma. ASTN2 protein may contribute to the pathogenic mechanism and symptom onset of mental disorders.

Publications

[Original papers]

1. Onodera, K., Tsuno, Y., Hiraoka, Y., Tanaka, K., Maejima, T., Mieda, M. In vivo recording of the circadian calcium rhythm in Prokineticin 2 neurons of the suprachiasmatic nucleus. *Sci Rep* 13. 16974, 2023.
2. Kanaya, T., Ito, R., Morizawa, Y.M., Sasaki, D., Yamao, H., Hiraoka, Y., Tanaka, K., Matsui, K. Glial modulation of the parallel memory formation. *Glia* 71. 2401-2417, 2023.
3. 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* 165. 211-229, 2023.

Department of Pathological Cell Biology

Professor	Shigeomi SHIMIZU
Project Junior Associate Professor	Satoru TORII
Junior Associate Professor	Shinya HONDA
Project Junior Associate Professor	Masatsune TSUJIOKA
Assistant professor	Yoichi NIBE
Project Assistant Professor	Min Kyong SHIN

Our laboratory focuses on three main areas of research: 1) elucidation of the physiological and pathological significance of Golgi-membrane-associated degradation (GOMED), a cellular function that we have discovered, 2) analysis of cell death mechanisms and development of therapeutic agents for diseases derived from its failure, and 3) research on diseases originating from mitochondria and Golgi abnormalities. We aim to elucidate the essence of the operating principles of life based on these findings.

The main research this fiscal year was the development of a new visualization method for GOMED and detailed analysis of the molecular mechanism. For cell death, we identified non-adherent cell death (anoikis) that functions in individual mammals. As for organelle research, we elucidated the pathological mechanism of Parkinson's disease involving mitochondrial abnormalities.

〈**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 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. 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.

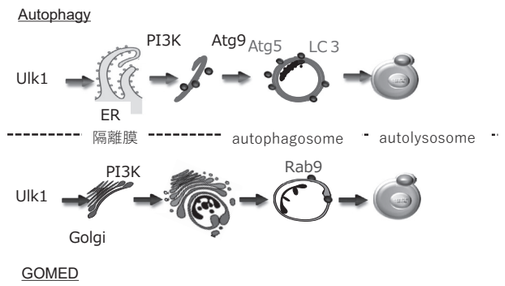


Figure 1. Hypothetical model of autophagy and GOMED.
Autophagy requires Atg5 and is originated from the ER membrane. In contrast, GOMED occurs independently of Atg5 and is originated from the Golgi membrane.

2, 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. Recently, we discovered a new execution mechanism of cell death by loss of cell adhesion (anoikis).

3, Disease research originating from organelle abnormalities

We analyzed the pathogenic mechanism of Parkinson's disease caused by *PARK22/CHCHCHD2* mutation, one of the familial Parkinson's diseases, and found that translocation of CHCHD2 from mitochondria and activation of casein kinase 1 (CK1) are involved. We also found that the disease severity is improved by inhibitors of casein kinase 1.

Highlights.

1) Development of a new method to visualize the progress of GOMED and autophagy

In collaboration with Dojin Chemical Research Institute, we developed three fluorescent reagents, DAPGreen, DAPRed, and DALGreen, and investigated in detail from which stage of GOMED and autophagy they are labeled. We found that (1) DAPGreen labels all structures with green fluorescence, (2) DAPRed labels structures after the middle stage with red fluorescence, and (3) DALGreen labels only structures in the late stage with green fluorescence. These compounds made it possible to measure the progress of GOMED and autophagy.

2) Elucidation of the pathogenic mechanism of Parkinson's disease caused by *Park22* genetic mutation

Using Neuro2a cells, we found that CHCHD2 is a protein originally localized in the mitochondria, but the mutant protein (CHCHD2^{T61I}) of familial Parkinson's disease localizes in the cytoplasm. We also found that this CHCHD2^{T61I} recruits CK1 ε / δ, which phosphorylates α-synuclein and induces neuronal cell death. These results were confirmed in Chchd2^{T61I} knock-in mice, patient iPS cell-derived dopaminergic neurons, and postmortem brains. In addition, treatment of cells and mice with CK1 ε / δ inhibitors suppressed α-synuclein phosphorylation and dopaminergic neuron cell death, and improved motor abnormalities in Chchd2^{T61I} knock-in mice (Figure 2).

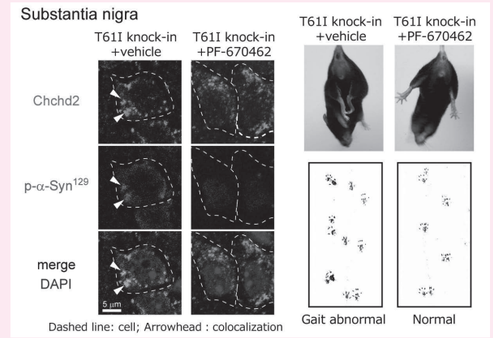


Figure 2. Phenotypic improvement with the CK1 ε / δ inhibitor. PF-670462

Publications

[Original paper]

1, Identification of a novel type of focal adhesion remodelling via FAK/FRNK replacement, and its contribution to cancer progression. *M. Tsujioka, K. Miyazawa, M. Ohmuraya, et al.* **Cell Death Dis.** 14, Article number: 256, 2023

2, Development of small fluorescent probes for the analysis of autophagy kinetics. *H. T. Sakurai, H. Iwashita, S. Arakawa, et al.* **iScience** 26, 107218, 2023

3, Involvement of casein kinase 1 epsilon/delta (Csnk1e/d) in the pathogenesis of familial Parkinson's disease caused by CHCHD2. *S. Torii, S. Arakawa, S. Sato, et al.* **EMBO. Mol. Med.** 15, e17451, 2023

4, Beclin1 is essential for the pancreas development. S. Mehanna, S. Arakawa, M. Imasaka;et al. **Developmental Biology.** 504, 113-119, 2023

Department of Neuroinflammation and Repair

Professor Takashi Shichita
Assistant Professor Seiichiro Sakai, Jun Tsuyama

Research outline

Stroke and dementia are major factors leading to the need for nursing care and bedridden conditions, and an increase in the number of patients worldwide is expected. However, since there are no therapeutic drugs to restore lost brain function in stroke and dementia, they are cited as representative examples of refractory diseases with few means of regaining lost brain function. When the brain is damaged, inflammation occurs, followed by the activation of neural repair programs within the brain, leading to a certain degree of brain function recovery. In our department, we are trying to develop therapeutic methods that enable the recovery of brain function by enhancing and sustaining the spontaneous recovery mechanisms in the brain.

Research project

Identification of brain-reparative lipids and brain-autonomous neural repair mechanisms through PLAG2E-dependent metabolism of phospholipids and polyunsaturated fatty acids.

Stroke is divided into ischemic stroke, where the brain's blood vessels are blocked and blood flow stops, and hemorrhagic stroke, where the brain's blood vessels rupture and bleed. In either case, when the stroke occurs, neuronal cells are damaged, leading to impairment of brain function. Although recovery of lost brain function occurs through rehabilitation after a stroke to some extent, it is believed that synaptic connections are newly formed within the brain, contributing to neural repair to compensate for lost brain function. Thus, the injured brain possesses the ability to repair itself to some extent. However, the mechanism by which surviving neuronal cells acquire repair capabilities after stroke has remained unclear.

When cells die due to tissue damage, intracellular proteins and other molecules are released, causing inflammation by directly activating immune cells. In the case of stroke, endogenous molecules released from dying brain cells trigger inflammation, leading to further neuronal damage. It has been discovered that the substances causing inflammation are resolved by being removed from the brain, leading to the resolution of inflammation and transitioning to neural repair. Furthermore, various lipid metabolites synthesized after tissue damage are known to be involved in the control of inflammation, with lipid metabolites reported to either promote or inhibit inflammation. In this study, we elucidated the mechanism of generating important brain lipid metabolites crucial for neural repair after brain injury.

First, using a mouse model of brain ischemia, we comprehensively analyzed the presence of lipid metabolites in the brain from the onset of cerebral infarction to one week later. Lipids were extracted from tissues surrounding the cerebral infarction site, and using mass spectrometry, we examined the types and quantities of phospholipid metabolites and fatty acids. As a result, metabolites of dihomo-gamma-linolenic acid, an unsaturated fatty acid, were found to increase from three to six days after the onset of cerebral infarction. Subsequently, we analyzed whether the symptoms of cerebral infarction changed in mice lacking phospholipase A2 (PLA2) subtype, an enzyme that cleaves unsaturated fatty acids from phospholipids constituting cell membranes, through behavioral evaluations of mice and staining of brain tissues after ischemic stroke. The results showed that mice lacking one subtype of phospholipase A2, PLA2G2E, exhibited worsening neurological symptoms after cerebral infarction and an increase in infarct volume. Furthermore, a comparison of gene expression in the brain tissue surrounding the infarct lesion between wild-type mice and *Pla2g2e*-deficient mice revealed a decrease in the expression of genes involved in neural repair in the *Pla2g2e*-deficient mice.

We particularly observed a significant decrease in the expression of peptidyl arginine deiminase 4 (PADI4) in the peri-infarct lesion of PLA2G2E-deficient mice. We investigated the expression of PLA2G2E and PADI4 in the brain ischemia model mice and human ischemic stroke patients through immunohistochemistry. As a result, we found the expression of PLA2G2E and PADI4 in surviving neurons in the tissues surrounding the cerebral infarction site from three days to one week after the onset of cerebral infarction. PLA2G2E is an enzyme that is released into the extracellular space and works externally. When PLA2G2E is produced by neurons in the tissues surrounding the cerebral infarction site, it was thought to primarily metabolize phosphatidylserine from the membrane debris of dead neurons, producing metabolites of unsaturated fatty acids.

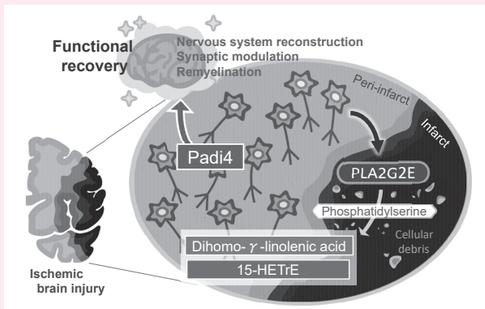
Since the level of dihomo-gamma-linolenic acid, one of the unsaturated fatty acids that increased after cerebral infarction, was decreased in *Pla2g2e*-deficient mice, it was anticipated that metabolites of dihomo-gamma-linolenic acid might be involved in the neural repair mechanism after cerebral infarction through PADI4. Therefore, dihomo-gamma-linolenic acid was orally administered to *Pla2g2e*-deficient mice with ischemic stroke. As a result, an increase in the expression of PADI4 was observed in neurons surrounding the infarct lesion, along with improvement in neurological symptoms after cerebral infarction and reduced infarct volume. Furthermore, when various fatty acid metabolites were added to the culture medium of neuronal cell lines to investigate whether the expression of PADI4 would change, the addition of one of the metabolites of dihomo-gamma-linolenic acid, 15-hydroxyeicosatrienoic acid (15-HETrE), significantly increased the expression of PADI4. Intravenous administration of 15-HETrE to ischemic stroke model mice resulted in an increase in the expression of PADI4 and genes related to neural repair in neurons surrounding the cerebral infarction site, leading to improvement in neurological symptoms after brain ischemia and reduction in infarct volume. From these results, it was demonstrated that dihomo-gamma-linolenic acid and its metabolite, 15-HETrE, have the ability to promote neural repair through PADI4.

Until now, it has been known that neural repair after brain injury leads to some degree of recovery in brain function, but the molecular mechanism by which neural repair is initiated has remained unclear. In this study, we discovered a novel neural repair mechanism where fatty acid metabolites generated after cerebral infarction trigger neural repair and induce gene expression related to neural repair through the citrullination of histones by PADI4. Through this research, we were able to demonstrate for the first time that in addition to controlling inflammation, lipid metabolites in the brain play an important role in neural repair after tissue damage. Dihomo-gamma-linolenic acid and 15-HETrE, reparative lipids that induce the expression of PADI4 in neurons, were found to promote neural repair and improve neurological symptoms after cerebral infarction. These neuroreparative lipids are obtainable through diet, and they hold promise for application in novel therapies aimed at improving functional outcomes after stroke.

Highlight

With brain tissue damage, inflammation is initiated in the brain, leading to brain dysfunction. However, subsequent spontaneous recovery of neurological symptoms suggests that the brain possesses certain repair functions. Phospholipase A2 subtype PLA2G2E, produced by neurons surrounding infarct lesion, metabolizes phosphatidylserine included in cellular debris, generating neuroreparative lipids such as dihomo-gamma-linolenic acid and 15-HETrE. While these lipids were previously unknown in brain function, this study revealed that inducing the expression of the transcription-regulating factor PADI4 in surviving neurons surrounding infarct lesion leads to the expression of neural-repair-associated genes necessary for neural tissue reconstruction, synaptic formation, and remyelination.

Since dihomo-gamma-linolenic acid and 15-HETrE can be obtained through diet, their application in dietary therapy to promote functional recovery after brain injury holds promise.



Publications

1) Nakamura A, Sakai S, Taketomi Y, Tsuyama J, Miki Y, Hara Y, Arai N, Sugiura Y, Kawaji H, Murakami M, Shichita T. PLA2G2E-mediated lipid metabolism triggers brain-autonomous neural repair after ischemic stroke. ***Neuron.*** 111(19):2995-3010 (2023)
2) Shichita T, Ooboshi H, Yoshimura A. Neuroimmune mechanisms mediating post-ischemic brain injury and repair. ***Nat Rev Neurosci.*** 24(5):299-312 (2023)
3) Koyama R, Shichita T. Glial roles in sterile inflammation after ischemic stroke. ***Neurosci Res.*** 187:67-71 (2023)

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 Drug Discovery and Design

【Existent Departments】

Structural Biology
Functional Genomics
Genomic Function and Diversity
Computational and Systems Biology
Advanced Nanomedical Engineering

【Main Research Activities】

Press releases in 2023

- Development of a cell co-localization network analysis tool called "DeepCOLOR" that utilizes deep generative models. 〔*Department of* Computational and Systems Biology〕
- Unraveling the mechanism of stem cell differentiation control to preserve gastric mucosa. 〔*Department of* Functional Genomics〕
- Establishment of high-purity encapsulation mRNA vaccine manufacturing technology, the PureCap method. 〔*Department of Advanced Nanomedical Engineering*〕

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. Binding Mechanism between HLA-A*24:02 and Modified Wilms' Tumor 1 Peptide

The Human Leukocyte Antigen (HLA) system is a complex group of genes that play a central role in the immune system. The HLA genes belonging to this system are essential for regulating immune responses through the important process of antigen presentation on the cell surface. Specifically, these genes are involved in coding for Major Histocompatibility Complex (MHC) class I and class II molecules, determining how cells present antigenic peptides on their surface and are surveilled by the immune system. MHC class I molecules are present on all nucleated cells and enhance surveillance by $CD8^+$ T cells (cytotoxic T cells), especially presenting antigens from internal sources (viruses or antigens derived from cancer cells). The diversity of HLA genes is very high, and the genetic background of an individual significantly influences immune responses. In particular, the HLA-A*24:02 allele, which is frequently observed in Asian populations, is one of the alleles that draw particular attention within this genetic diversity. Research related to HLA-A24 has revealed its relationship with autoimmune diseases, infectious diseases, and cancer, and identifying peptides that specifically bind to this allele provides crucial information for designing immunotherapies. Specifically, identifying peptides secreted by cancer cells and targeting them for vaccines is expected to induce T cells that attack cancer cells.

In this study, we performed a crystallographic study of the complex structure between the modified Wilms' Tumor 1 (mWT1) peptide and the HLA-A*24:02 allele. Using the automatic data collection system at the synchrotron facility SPring-8, we acquired diffraction data. Based on this data, we elucidated the structure of the complex at a resolution of 2.48 Å (Figure 1). Detailed affinity and structural characteristics of the binding between the mWT1 peptide and HLA-A24 were revealed, which holds significant implications for the design of high-affinity peptides targeting HLA-A24 for cancer immunotherapy and for understanding the binding mechanisms to different MHC class I molecules.

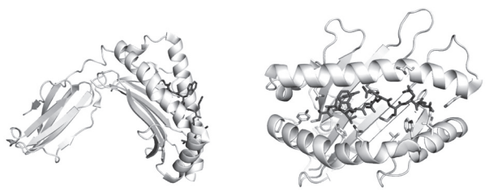


Fig.1 (left) Crystal structure of HLA-A24 and mWT1 peptide complex. (right) Close up view of the interaction between HLA-A24 and mWT1 peptide.

This work is performed in collaboration with Professor Narutoshi Kamiya at University of Hyogo, Professor Masayuki Oda at Kyoto Prefectural University, Associate Professor Haruo Kozono at Tokyo University of Science, and Lecture Gert-Jan Bekker at Osaka University.

2. Functional analysis of SLC26A9 using MD simulation

Solute Carrier Family 26 member A9 (SLC26A9) is a Cl^- transporter that is primarily expressed in the epithelial cells of the lungs and stomach, contributing to mucociliary clearance and the production of gastric acid. SLC26A9 interacts with the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) to regulate the permeability of Cl^- ions. Cystic fibrosis is known as a genetic disease commonly found in Caucasians, and currently, there are no effective treatments available. Due to the co-localization and functional correlation between SLC26A9 and CFTR, SLC26A9 is considered a potential therapeutic target for restoring Cl^- ion transport in the epithelial cells of patients with cystic fibrosis. SLC26A9 is mainly composed of 14 transmembrane helices and a Sulphate Transporter and Anti-Sigma factor antagonist (STAS) domain located on the cytoplasmic side, with a motif at its C-terminus that binds to the PDZ domain. Although the flexibility of the transmembrane helices is suggested to be involved in transport activity through interaction with the substrate, the transport mechanism of SLC26A9 is not yet fully understood. Furthermore, mutations in the STAS domain affect the interaction between transmembrane regions, leading to a decrease in transport function, while the role of the STAS domain in Cl^- ion transport is largely unknown.

In this study, molecular dynamics (MD) simulations were performed on the full-length model of human SLC26A9, a model lacking the STAS domain (ΔSTAS), and a model lacking the C-terminus (ΔC). The results of the MD simulations showed that the TM helix 12 interacts with the STAS domain through salt bridges, changing from a bent structure to an extended structure, which leads to the stable binding of Cl^- ions (Figure 2). Additionally, the large asymmetrical movements of the STAS domain were suggested to induce structural changes in the transmembrane region, promoting Cl^- ion transport. The insights gained from this study not only deepen the understanding of function of SLC26A9 but also represent an important step towards the rational design of new drugs and more effective treatments for diseases related to Cl^- ion transport, especially cystic fibrosis.

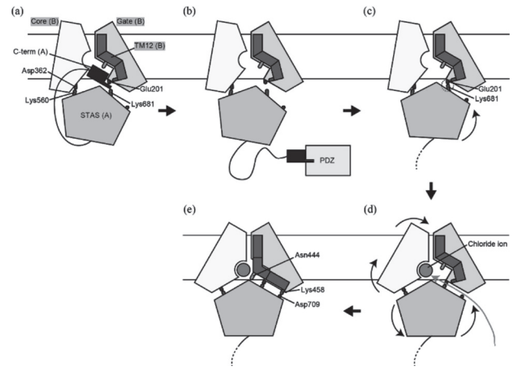


Fig.2 Schematic view of the transport mechanism of SLC26A9.

This work is performed in collaboration with Professor Kengo Kinoshita and Associate Professor Hafumi Nishi at Tohoku University, and Lecture Satoshi Omori at Nagahama Institute of Bio-Science and Technology.

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. Bekker GJ, Numoto N, Kawasaki M, Hayashi T, Yabuno S, Kozono Y, Shimizu T, Kozono H, Ito N, Oda M, Kamiya N. Elucidation of binding mechanism, affinity and complex structure between mWT1 tumor-associated antigen peptide and HLA-A*24:02. Protein Science, 32, e4775, 2023.
2. Kamegawa A, Suzuki S, Suzuki H, Nishikawa K, Numoto N, Fujiyoshi Y. Structural analysis of the water channel AQP2 by single-particle cryo-EM. J. Struct. Biol., 215, 107984, 2023.
3. Kozai D, Numoto N, Nishikawa K, Kamegawa A, Kawasaki S, Hiroaki Y, Irie K, Oshima A, Hanzawa H, Shimada K, Kitano Y, Fujiyoshi Y. Recognition mechanism of a novel gabapentinoid drug, mirogabalin, for recombinant human $\alpha 2\delta 1$, a voltage-gated calcium channel subunit. J. Mol. Biol., 435, 168049, 2023.

Department of Functional Genome Informatics

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

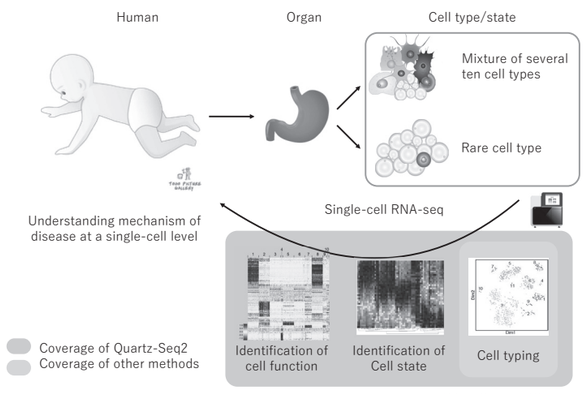
Research Objects

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

Mechanisms of Stem Cell Differentiation Control that Protect the Gastric Mucosa Uncovered

We have elucidated the mechanisms that maintain the undifferentiated state of gastric stem cells and the mechanisms that lead to their differentiation into surface mucous cells that secrete mucus to protect the surface of the stomach[4]. In this study, we used the world's highest precision single-cell RNA sequencing method, "Quartz-Seq2," developed by our team, to precisely analyze the gene expression of various cells composing the mouse stomach at the single-cell level. Using this gene expression analysis data, it became possible to predict the signal transduction pathways that are activated during the differentiation process of gastric stem cells. Additionally, by adding predicted activators or inhibitors of these signal pathways to three-dimensionally cultured gastric stem cells and verifying their effects, we successfully uncovered a part of the mechanism that maintains homeostasis, ensuring the stomach functions normally. This research is expected to contribute to the understanding of the mechanisms of development of gastric cancer and its precancerous lesions, metaplasia, which are caused by the disruption of stem cell regulatory mechanisms.



Research achievements

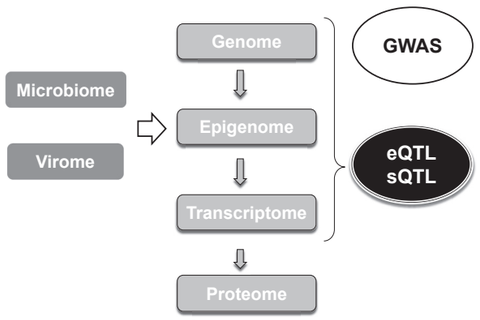
1. Koki Tsuyuzaki, Manabu Ishii, Itoshi Nikaido. Sctensor detects many-to-many cell-cell interactions from single cell RNA-sequencing data. BMC bioinformatics, 24(1), 2023, 420-420
2. Yasuyuki Shima, Henrik Skibbe, Yohei Sasagawa, Noriko Fujimori, Yoshimi Iwayama, Ayako Isomura-Matoba, Minoru Yano, Takumi Ichikawa, Itoshi Nikaido, Nobutaka Hattori, Tadafumi Kato. Distinctiveness and continuity in transcriptome and connectivity in the anterior-posterior axis of the paraventricular nucleus of the thalamus. Cell reports, 42(10), 2023, 113309-113309,
3. Nao Ogura, Yohei Sasagawa, Tasuku Ito, Toshiaki Tameshige, Satomi Kawai, Masaki Sano, Yuki Doll, Akira Iwase, Ayako Kawamura, Takamasa Suzuki, Itoshi Nikaido, Keiko Sugimoto, Momoko Ikeuchi. WUSCHEL-RELATED HOMEOBOX 13 suppresses de novo shoot regeneration via cell fate control of pluripotent callus Science Advances, 9(27), 2023,
4. Hitomi Takada*, Yohei Sasagawa*, Mika Yoshimura, Kaori Tanaka, Yoshimi Iwayama, Tetsutaro Hayashi, Ayako Isomura-Matoba, Itoshi Nikaido†, Akira Kurisaki†. Single-cell transcriptomics uncovers EGFR signaling-mediated gastric progenitor cell differentiation in stomach homeostasis. Nature communications, 14(1), 2023, 3750-3750,
5. Koki Tsuyuzaki, Kentaro Yamamoto, Yu Toyoshima, Hirofumi Sato, Manami Kanamori, Takayuki Teramoto, Takeshi Ishihara, Yuichi Iino, Itoshi Nikaido. WormTensor: a clustering method for time-series whole-brain activity data from C. elegans. BMC bioinformatics, 24(1), 2023, 254-254,
6. Koki Tsuyuzaki, Itoshi Nikaido. nnTensor: An R package for non-negative matrix/tensor decomposition Journal of Open Source Software, 2023,
7. Hiromi Nishimura, Yayoi Ikawa, Eriko Kajikawa, Natsumi Shimizu-Mizuno, Sylvain Hiver, Namine Tabata-Okamoto, Masashi Mori, Tomoya Kitajima, Tetsutaro Hayashi, Mika Yoshimura, Mana Umeda, Itoshi Nikaido, Mineo Kurokawa, Toshio Watanabe, Hiroshi Hamada. Maternal epigenetic factors in embryonic and postnatal development. Genes to cells : devoted to molecular & cellular mechanisms, 2023

Department of Genomic Function and Diversity

Professor **Yuta Kochi**
Associate professor **Nao Nishida**
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.

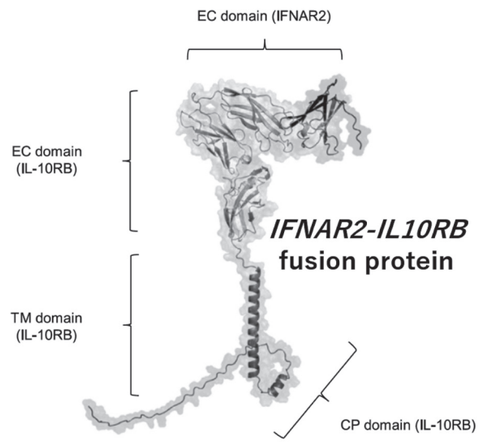


Research activities

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 (i^2r-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). Utilizing this Atlas, we identified an IFNAR2-IL10RB fusion transcript isoform involved in the exacerbation of COVID-19 (*Immunity* 2023). Thus, analysis focusing on alternative splicing will advance the understanding of various immunological conditions.



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 : Chiu Shu Chun (Technical staff)

Publications

Original articles

1. Chang S, Torii S, Inamo J, Ishikawa K, Kochi Y, et al. Uncovering the Localization and Function of a Novel Read-Through Transcript 'TOMM40-APOE'. ***Cells.*** 13. 2023.
2. Fujitani H, Eguchi H, Kochi Y, Arai T, Muramatsu M, et al. Rare germline variants in pancreatic cancer and multiple primary cancers: an autopsy study. ***Eur J Cancer Prev.*** 32. 286-97, 2023.
3. Iwasaki Y, Takeshima Y, Nakano M, Okubo M, Ota M, et al. Combined plasma metabolomic and transcriptomic analysis identify histidine as a biomarker and potential contributor in SLE ***pathogenesis. Rheumatology (Oxford).*** 62. 905-13, 2023.
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5. Ono C, Tanaka S, Myouzen K, Iwasaki T, Ueda M, et al. Upregulated Fcrl5 disrupts B cell anergy causes autoimmune disease. ***Front Immunol.*** 14. 1276014, 2023.
6. Otomo N, Khanshour AM, Koido M, Takeda K, Momozawa Y, et al. Evidence of causality of low body mass index on risk of adolescent idiopathic scoliosis: a Mendelian randomization study. ***Front Endocrinol*** (Lausanne). 14. 1089414, 2023.
7. Takada K, Ueda MT, Shichinohe S, Kida Y, Ono C, et al. Genomic diversity of SARS-CoV-2 can be accelerated by mutations in the nsp14 gene. ***iScience.*** 26. 106210,

Review article

1. Kochi Y. Genetics of rheumatoid arthritis (in Japanese). ***Internal Medicine.*** 112(10), 2023.

Department of Computational and Systems Biology

Professor	Teppei Shimamura
Associate Professor	Shuto Hayashi
Project Lecturer	Ko Abe
Assistant Professor	Haruka Hirose
Technical Support Staff	Yoko Oka, Mitsuki Narita, Mayumi Nishio, Mai Yamada

Research Overview

In the field of Computational Systems Biology, we conduct research from both theoretical and practical perspectives on data-driven methodologies that comprehensively capture diseases from a systemic viewpoint by leveraging state-of-the-art bioinformatics, data science, and deep learning techniques to analyze data. By unraveling the underlying principles of biological phenomena as systems from multi-omics data (genomics, epigenomics, transcriptomics, proteomics, metabolomics, etc.) and in vivo imaging data obtained from next-generation sequencers, we aim to elucidate disease mechanisms and pathologies, identify disease biomarkers, achieve high-precision prediction of therapeutic effects, and discover innovative therapeutic targets.

Specifically, we conduct research on the following three themes to understand life through the elucidation and modeling of biological control systems.

Omics Analysis

We investigate the operating principles of biological systems from the bottom-up using comprehensive molecular information (omics data) ranging from genomics, epigenomics, transcriptomics, proteomics, to metabolomics, and link this to elucidating the pathophysiology of diseases such as cancer, infectious diseases, and psychiatric disorders. Recently, we have been developing unique analysis techniques tailored to the latest measurement technologies, such as simultaneous measurement of multiple modalities of omics at the single-cell level (single-cell multi-omics analysis) and transcriptome measurement technology with spatial information (spatial transcriptomics analysis).

Imaging Analysis

We analyze time-evolving video image information (imaging data) that measures the interaction networks between tissues, cells, and biomolecules using deep learning and statistical models. We conduct research to mathematically quantify, visualize, and model the dynamics of these networks.

Drug Discovery and Structural Analysis

To understand intracellular reactions and biological functions, we investigate molecular interactions at the molecular level by analyzing molecular structures, leading to drug discovery (control of biological reactions, molecular design) to improve various disease symptoms. In particular, by integrating theoretical scientific approaches (molecular dynamics, quantum chemistry, molecular docking) with information analysis (topological data analysis, statistical analysis, machine learning), we are working on developing new technologies to clarify the relationship between 'structure and function'.

Research Highlights

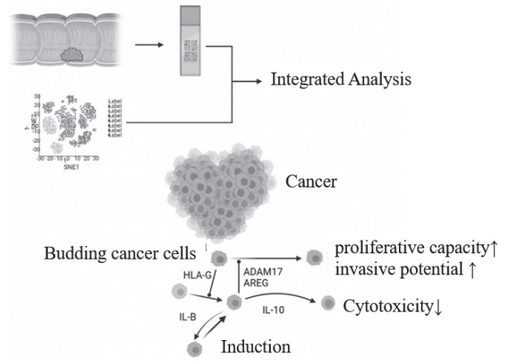
The research achievements in 2023 are as follows:

1. Development of a unified matrix factorization model for multidimensional omics data analysis without restrictions on data format or structure

We developed a new statistical framework called unified nonnegative matrix factorization (UNMF) to extract useful patterns from multidimensional omics data. The features of UNMF are as follows:

1. It can handle diverse data structures and formats in a unified manner without being limited by the format or structure of the data.
2. It can be seamlessly applied to tensor data, including missing values and repeated measurements.
3. We developed an efficient learning algorithm using the variational Bayesian method.

To demonstrate the usefulness of the proposed method, we showed some application examples to multidimensional omics data. In metagenome analysis, we showed that the proposed method can effectively identify differences between cases and controls. In the example of gene expression, we showed that the proposed method can make reasonable predictions while maintaining interpretability.



Stromal response by clustered colorectal cancer cells

2. Development of analysis technology for changes in protein structure and inter/intra-molecular interactions

We developed a new analysis framework called "DAIS" to analyze changes in protein structure and inter/intra-molecular interactions due to amino acid mutations. The proposed method combines molecular dynamics simulation (MD) and persistent homology, a topological data analysis (TDA) technique, enabling the following:

1. Detection of structural changes and hydrogen bond changes associated with amino acid mutations.
2. Analysis of the diversity of protein-protein interactions that change due to amino acid mutations.

We compared the SARS-CoV-2 spike protein mutants and wild-type and extracted the structural and hydrogen bond changes caused by the D614G mutation. We also compared the binding structures of the spike protein receptor-binding domain (RBD) and ACE2 receptor in mutants such as BA.1, BA.2, BA.2.75, and BA.5, and clarified the diversity of binding due to mutation patterns. This method is a powerful tool for predicting the impact of amino acid mutations on protein structure and function. For example, in viral infection, it may be possible to predict changes in pathogenicity due to mutations, and in drug target proteins, it may be possible to predict the emergence of drug resistance due to mutations.

3. Elucidation of information exchange at the single-cell level in the tumor microenvironment of the invasive front of colorectal cancer

In this study, we performed an integrated analysis using single-cell RNA-seq data and spatial transcriptome data from Asian colorectal cancer patients. We showed that HLA-G molecules derived from cancer cells are involved in the induction of SPP1+ macrophages, which are closely related to tumor malignancy. Furthermore, we confirmed the reproducibility of these analysis results by immunohistochemical staining of 20 colorectal cancer specimens and transplantation experiments of colorectal cancer HLA-G knockout cells into mice. This discovery proposes a therapeutic approach targeting malignant macrophages, known as the second immune checkpoint, and is expected to lead to a new therapeutic approach that contributes to the reduction of colorectal cancer mortality.

Publications

Original Papers

1. Fujino S, et al.: Metastases and treatment-resistant lineages in patient-derived cancer cells of colorectal cancer. Commun Biol. 6, 1191, 2023
2. Li M, et al.: FXYD3 functionally demarcates an ancestral breast cancer stem cell subpopulation with features of drug-tolerant persisters. J Clin Invest. 133, e166666, 2023
3. Kato M, et al.: Acidic extracellular pH drives accumulation of N1-acetylspermidine and recruitment of protumor neutrophils. PNAS Nexus. 2, pgad306, 2023
4. Nakahara R, et al: Hypoxia activates SREBP2 through Golgi disassembly in bone marrow-derived monocytes for enhanced tumor growth. EMBO J. 42, e114032, 2023
5. Abe K, Shimamura T: UNMF: a unified nonnegative matrix factorization for multi-dimensional omics data. Brief Bioinform. 24, bbad253, 2023
6. Takeuchi S, et al.: Plucked scalp hair follicle samples are useful RNA sources for mRNA analysis of most genodermatosis-associated genes. J Dermatol Sci. 111, 68-70, 2023
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8. Kitagawa A, et al.: Convergent genomic diversity and novel BCAA metabolism in intrahepatic cholangiocarcinoma. Br J Cancer. 128, 2206-2217, 2023
9. Torii S, et al: Increased flexibility of the SARS-CoV-2 RNA-binding site causes resistance to remdesivir. PLoS Pathog. 19, e1011231, 2023
10. Ozato Y, et al: Spatial and single-cell transcriptomics decipher the cellular environment containing HLA-G+ cancer cells and SPP1+ macrophages in colorectal cancer. Cell Rep. 42, 111929, 2023

Books and Reviews

1. Shimamura T, Kojima Y: Analysis of cell state transition fluctuations using variational autoencoders and its application to mibyou research, Seitai no Kagaku (The Journal of Physiology) Vol.74 No.2 April 2023, 118-122.
2. Shimamura T: Latest technologies for single-cell multimodal analysis - Approaches for integrating modalities of the same cell and different cells, Experimental Medicine Special Issue Vol.41 No.15, 2423-2329.
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Department of Advanced Nanomedical Engineering

Professor Satoshi Uchida
Lecturer Yuki Mochida

Messenger RNA (mRNA) vaccines have proven highly effective and safe against COVID-19, with billions of doses administered to humans within just one year of emergency approval. This success has spurred intensive research and development in mRNA vaccines and therapeutics across various medical fields. These include infectious disease vaccines, cancer vaccines and immunotherapy, treatments for single-gene disorders, regenerative medicines, and genome editing. These applications require nano-sized drug delivery systems (nano DDS) for targeted mRNA delivery to specific tissues and cells. Our research focuses broadly on the nano DDS development and its therapeutic applications.

1. Nano DDS development

Our nano DDS utilizes polymeric micelles (PMs) coated with poly(ethylene glycol) (PEG). PMs prevent mRNA recognition by innate immune receptors in cells (**Fig. 1**), enabling mRNA delivery with minimal inflammatory responses. However, enhancing nuclease stability remains a challenge for PMs.

Current nano DDS development primarily concentrate on designing lipids and polymers. Alternatively, Prof. Uchida has pioneered nano DDS development centered on mRNA designs. Conventional methods directly modify mRNA molecules, for example by pseudouridine. Nonetheless, this strategy can compromise mRNA translational activity, limiting modification options. In contrast, our mRNA engineering approach involves hybridizing mRNA with RNA oligonucleotides bearing chemical moieties that stabilize nano DDS (**Fig. 2**). This strategy successfully enhances PM stability by introducing cholesterol moieties to mRNA, crosslinking mRNA and polycations in environment-responsive manners, and providing mRNA with steric structures (***Adv Drug Deliv Rev*** 199, 114972, (2023)).

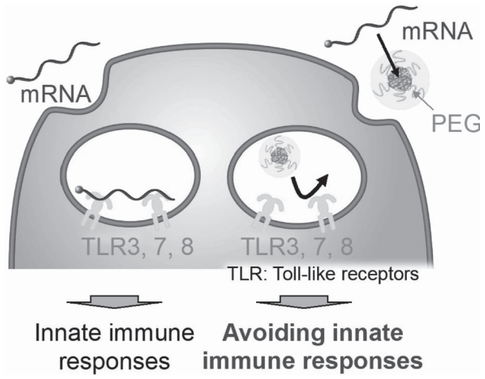


Fig. 1 Polymeric micelles

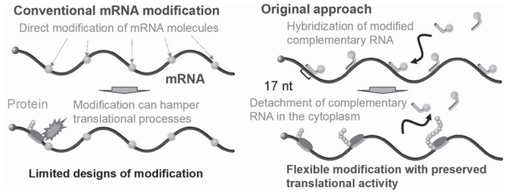


Fig. 2 mRNA engineering

2. Applications of mRNA vaccines and therapeutics

1) Infectious disease vaccines

A challenge of current mRNA vaccines based on lipid nanoparticles (LNPs) is their relatively high reactogenicity. While such reactogenicity is acceptable for a few doses in pandemic, safer vaccine platforms are needed for repeated boosting against COVID-19 and for other infectious diseases. To address this, we are developing carrier-free naked mRNA vaccines. For efficient vaccination using naked mRNA, we targeted the dermal tissue rich in antigen-presenting cells and used a jet injector to improve the delivery efficiency. This strategy achieved efficient antibody production and protective effects against SARS-CoV-2 challenge in mice, without systemic adverse effects. This vaccine was also effective in non-human primates.

2) Cancer vaccines

Unlike infections disease vaccines that require safety improvement, the primary challenge with cancer vaccines is enhancing their efficacy. The challenge stems from the low antigenicity of self-derived tumor antigens and the immunosuppressive tumor microenvironment. To boost vaccination efficacy, we employ mRNA engineering, introducing immunostimulatory adjuvant functionalities to mRNA (***PNAS*** 120, e2214320120, (2023)). By hybridizing mRNA with immunostimulatory double-stranded RNA (dsRNA) targeting RIG-I, an innate immune receptor, we prepared comb-structured mRNA (**Fig. 3**). This approach enables controlling immunostimulation intensity by adjusting the number of dsRNA strands, preventing excessive inflammatory responses. In mouse experiments, the comb-structured mRNA improved antigen-specific cytotoxic T lymphocyte (CTL) activity of lipid-based systems used in clinical trials, showing efficient anti-cancer responses against melanoma by targeting a tumor-associated antigen. This system also enhanced the CTL responses of other vaccine delivery systems, such as PM and LNP used in an approved COVID-19 vaccine.

3) Protein replacement therapy and genome editing

Apart from vaccine applications, mRNA offers promise in expressing therapeutic proteins and inducing genome editing. In these applications, immunostimulation induced by mRNA delivery systems can cause adverse effects and hinder disease treatment in the target tissue. Here, the low immunostimulatory properties of PMs is beneficial. Indeed, using PMs, in animal models, we succeeded in treating fulminant hepatitis using anti-apoptotic mRNA, brain ischemia and spinal cord injury using neurotrophic factor mRNA, Alzheimer's disease using mRNA encoding single-chain antibody against amyloid β, and osteoarthritis and intervertebral disc disease using chondrogenic mRNA. Moreover, RNA-based delivery of CRISPR/Cas9 using PMs has effectively induced in vivo genome editing in the mouse brain.

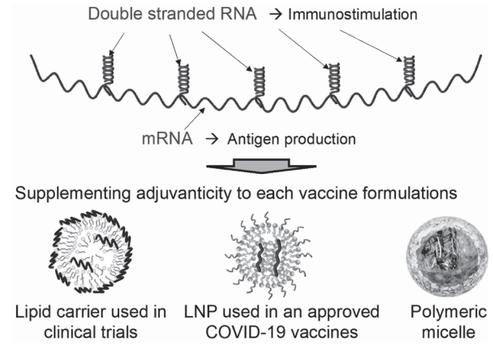


Fig. 3 Comb-structured mRNA for cancer vaccines

Publications

M Inagaki, N Abe, Z Li, Y Nakashima, S Acharyya, K Ogawa, D Kawaguchi, H Hiraoka, A Banno, Z Meng, M Tada, T Ishida, P Lyu, K Kokubo, H Murase, F Hashiya, Y Kimura, S Uchida, H Abe, Cap analogs with a hydrophobic photocleavable tag enable facile purification of fully capped mRNA with various cap structures, ***Nature communications*** 14, 2657, (2023)

T A Tockary, S Abbasi, M Matsui-Masai, A Hayashi, N Yoshinaga, E Boonstra, Z Wang, S Fukushima, K Kataoka, S Uchida, Comb-structured mRNA vaccine tethered with short double-stranded RNA adjuvants maximizes cellular immunity for cancer treatment, ***Proceedings of the National Academy of Sciences*** 120, e2214320120, (2023)

M Oba, M Shibuya, Y Yamaberi, H Yokoo, S Uchida, A Ueda, M Tanaka, An Amphipathic Structure of a Dipropylglycine-Containing Helical Peptide with Sufficient Length Enables Safe and Effective Intracellular siRNA Delivery, ***Chemical and Pharmaceutical Bulletin*** 71, 250-256, (2023)

W Yang, T Miyazaki, Y Nakagawa, E Boonstra, K Masuda, Y Nakashima, P Chen, L Mixich, K Barthelmes, A Matsumoto, P Mi, S Uchida, H Cabral, Block catiomers with flanking hydrolyzable tyrosinate groups enhance in vivo mRNA delivery via pi-pi stacking-assisted micellar assembly, ***Sci Technol Adv Mater*** 24, 2170164, (2023)

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, Shutong Fu, Hayato Kano, Rikuto Fukuhara**
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.

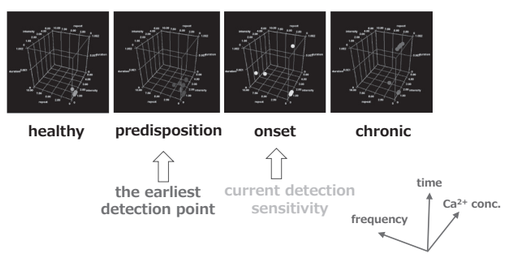


Figure 1. Ultrasensitive health monitoring system
Intravital imaging of Ca^{2+} signaling in immune cells using lineage-specific calcium biosensor mice allow to detect subtle predisposing abnormalities prior to pathogenesis, based on normal conditions. With this system, the progression from the onset 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

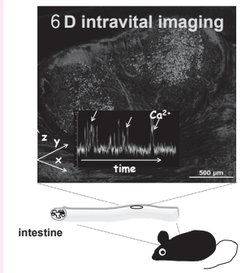
We aim to develop foods and medicines that target the predisposition of diseases, as well as foods and medicines that enhance physical and mental health. We evaluate the effects of foods, natural products, and their components on the immune system, nervous system, and intestinal epithelium including the endocrine. 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 medicines 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, calcium 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



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

Kawamoto S, Uemura K, Hori N, Takayasu L, Konishi Y, Katoh K, Matsumoto T, Suzuki M, Sakai Y, Matsudaira T, Adachi T, Ohtani N, Standley DM, Suda W, Fukuda S, Hara E. Bacterial induction of B cell senescence promotes age-related changes in the gut microbiota. Nat Cell Biol. 2023 Jun;25(6):865-876. doi: 10.1038/s41556-023-01145-5.

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, Tadahide Noguchi

Research Outcome

Tongue cancer accounts for approximately 60% of all oral cancers, the five-year survival rate is extremely low at 42% for advanced cancer, and 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.

To elucidate 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. For esophageal squamous cell carcinoma organoids, gene expression analysis also identified a pathway that is likely to be involved in chemotherapeutic drug resistance. We have generated chemotherapeutic drug-resistant cancer organoid lines lacking a transcription factor critical for chemotherapeutic drug resistance and are testing their drug sensitivity.

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.

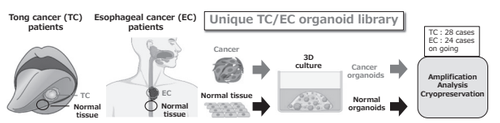


Fig.1 Establishment of human tongue cancer/esophageal cancer organoid library

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.

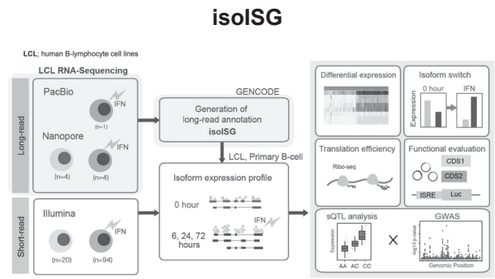
Research Outcome

1. Construction of splicing isoform catalogue

Type I interferon (IFN) is an important inflammatory cytokine in innate immunity. Using B cells stimulated with IFN-α, we conducted comprehensive expression analysis (RNA-seq) by long-read sequencing and created a isoform catalog, named isoISG (Ueda MT et al., manuscript in submission). It is expected that elucidation of the pathogenesis of various diseases related to innate immunity will progress by using this catalog.

2. Splicing isoform analysis in diseases

Functional analysis of the *TOMM40-APOE* fusion transcript identified in the risk gene locus of Alzheimer's disease confirmed that the protein translated by this isoform is localized in mitochondria and revealed that it induces cell death (Chang S et al., Cells 2023).



Publications

1. Chang S, Torii S, Inamo J, Ishikawa K, Kochi Y, Shimizu S. Uncovering the Localization and Function of a Novel Read-Through Transcript 'TOMM40-APOE'. ***Cells.*** 2023;13(1):69.

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 alpha-synuclein and aggresomes were observed in a PARK22/CHCHD2 mutant expressing cells. Moreover, casein kinase 1 epsilon was found to be a kinase that was bound to the CHCHD2 mutant and phosphorylated alpha-synuclein. Knock-in mice with the PARK22 gene mutation have been generated and analyzed. The mice showed abnormalities in locomotion and increased phosphorylated alpha-synuclein and aggresomes in the midbrain substantia nigra of mice.

Result2 : We identified PF-670462, an inhibitor of casein kinase 1 epsilon/delta, as a compound which can alleviate the abnormalities caused by the PARK22 gene mutation. The compound improved cellular abnormalities in cells, locomotor function and neuronal loss in knock-in mice. The improvements were also observed in patient iPS cell-derived neurons. These results were reported in the original paper 1.

Result3 : We have analyzed whether the PARK22 gene is also associated with the development of other familial Parkinson's diseases by using iPS cell-derived neurons.

Publications

[Original Paper]
1. Torii S, Arakawa S, Sato S, Ishikawa KI, Taniguchi D, Sakurai HT, Honda S, Hiraoka Y, Ono M, Akamatsu W, Hattori N, Shimizu S. Involvement of casein kinase 1 epsilon/delta (Csnk1e/d) in the pathogenesis of familial Parkinson's disease caused by CHCHD2. ***EMBO Molecular Medicine***, 15, e17451 (2023)
2. Chang S, Torii S, Inamo J, Ishikawa K, Kochi Y, Shimizu S. Uncovering the Localization and Function of a Novel Read-Through Transcript 'TOMM40-APOE'. ***Cells***, 13, 69 (2023)

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.

Laboratory of Cytometry and Proteome Research

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

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

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

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 staff 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 controlling 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…5/15, 6/13 (Carl Zeiss)
Laser Capture Microdissection…9/19 (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 FACSAria Fusion), confocal laser scanning microscope (FV10i-DOC).

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 (until March 31, 2023).

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 overall use cases was 193 in the year of 2023.

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 obtained approval from ethic committee to allow us to accept existing samples via opt-out method for specimen deposit. 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 one laboratory on campus. We also undertake tests for mycoplasma contamination and we received a request from two laboratories on campus. We continued the preservation service for the biological samples using a large liquid nitrogen tank (Fig.2), which was requested by several laboratories on campus.

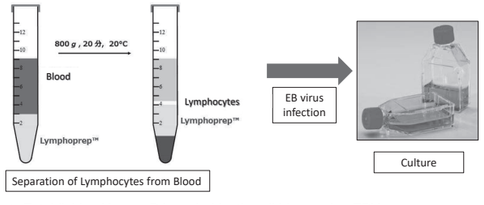


Fig. 1 Establishing Human B Lymphoblastic cell Lines using EBV

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.

This fiscal year, we introduced the long-read sequencer from Oxford Nanopore Technologies. We have developed a technology for sequencing single cells and bulk RNA from multiple specimens using long-read sequencing and Quartz-Seq2. In collaboration with the RIKEN, we also developed a method for analyzing cell-cell interactions from single-cell RNA sequencing data, and we have published the source code and a paper on this research.

In FY2023, four intra- and nine external collaborations (two of which was with a company) were conducted, and seven papers were published.

Research achievements:

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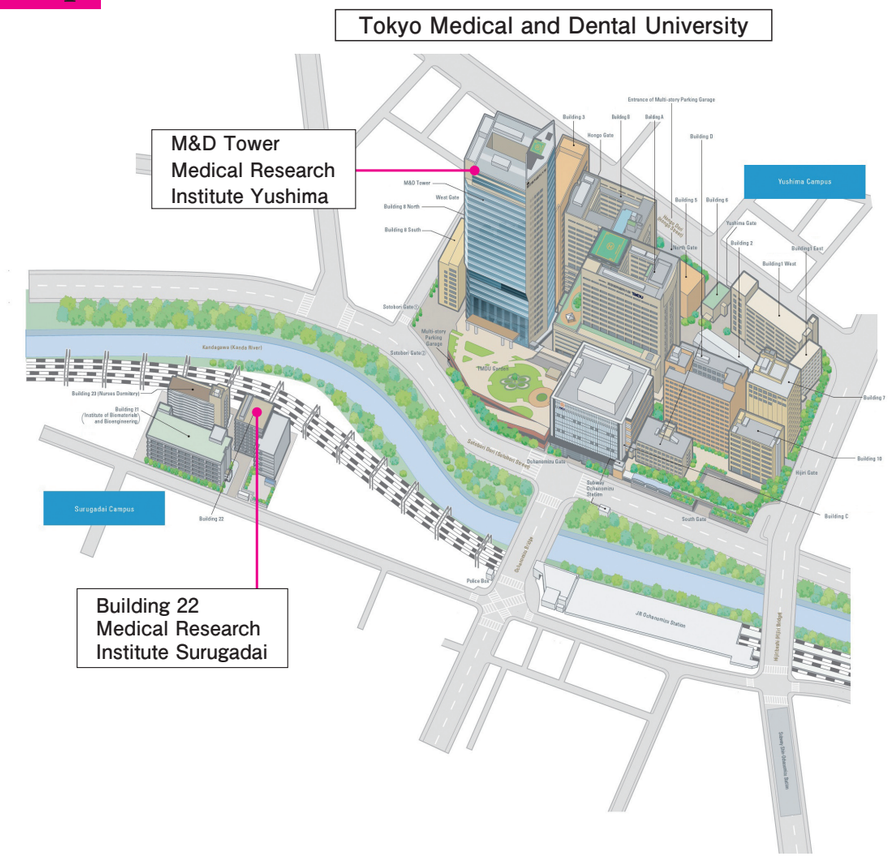
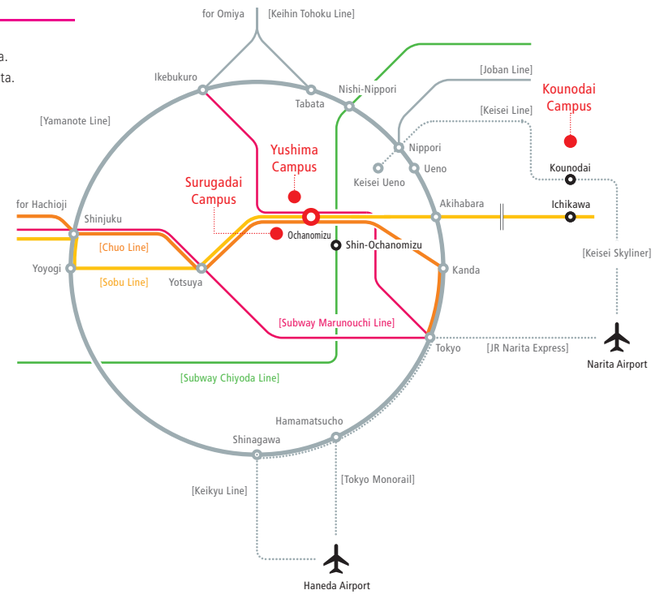
Access Map



Nearest Stations

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



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