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http://www.tmd.ac.jp/mri/english/index.html

ANNUAL REPORT 2022

Tokyo Medical and Dental University

Medical Research Institute / Tokyo Medical and Dental University

Annual Report 2022

2022

Annual Report

Medical Research Institute

Tokyo Medical and Dental University

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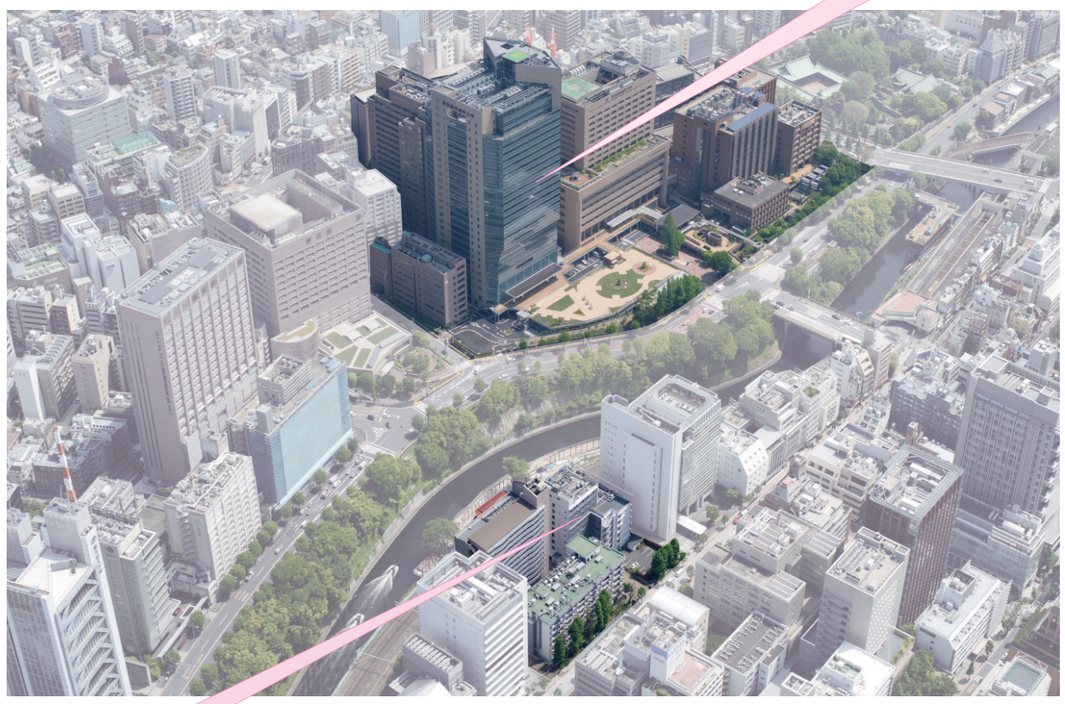
Yushima Area

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

Medical Research Institute

Department of Molecular Neuroscience, Department of Pathological Cell Biology, Department of Developmental and Regenerative Biology, Department of Immunology, Department of Molecular Cytogenetics, Department of Molecular Genetics, Department of Medical Science Mathematics, Department of Molecular Cell Biology, Department of Bio-informational Pharmacology, Department of Epigenetics, Department of Stem Cell Regulation, Department of Neuropathology, Department of Structural Biology, Department of Biodefense Research, Department of Stem Cell Biology, Department of Molecular Epidemiology, Department of Biochemical Pathophysiology, Department of Functional Genome Informatics, Department of Genomic Function and Diversity, Department of Medical Chemistry, Department of Biomolecular Pathogenesis, Frontier Research Unit, Intractable Disease Integrated Research Laboratory, Advanced Technology Laboratory, 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 (2021)

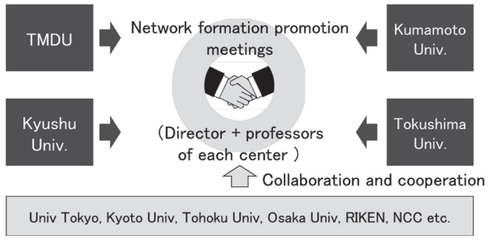
- Director: Hiroshi Nishina
- Deputy Director: Shigeomi Shimizu
- Faculty Meeting
- Research Divisions
  - Division of Advanced Molecular Medicine
    - Department of Molecular Medicine and Metabolism — (under consideration)
    - Department of Medical Chemistry — Professor Katsumori Segawa
    - Department of Molecular Cell Biology — Professor Hiroshi Shibuya
    - Department of Molecular Neuroscience — Professor Kohichi Tanaka
    - Department of Biodefense Research — Professor Toshiaki Ohteki
    - Department of Bio-informational Pharmacology — Professor Tetsushi Furukawa
    - Department of Stem Cell Regulation — Professor Tetsuya Taga
    - Department of Structual Biology — Professor Nobutoshi Ito
    - Frontier Research Unit Laboratory of Oxygen Biology — (under consideration)
  - Division of Pathophysiology
    - Department of Neuropathology — Professor Hitoshi Okazawa
    - Department of Biochemical Pathophysiology — Professor Takehiko Sasaki
    - Department of Pathological Cell Biology — Professor Shigeomi Shimizu
    - Department of Developmental and Regenerative Biology — Professor Hiroshi Nishina
    - Department of Stem Cell Biology — Professor Emi K. Nishimura
    - Department of Immunology — Professor Takeshi Tsubata
    - Department of Molecular Pathogenesis — (under consideration)
  - Division of Medical Genomics
    - Department of Molecular Cytogenetics — Professor Johji Inazawa
    - Department of Molecular Genetics — Professor Yoshio Miki
    - Department of Molecular Epidemiology — Professor Masaaki Muramatsu
    - Department of Functional Genome Informatics — Professor Itoshi Nikaido
    - Department of Genomic Function and Diversity — Professor Yuta Kochi
    - Department of Epigenetics — (under consideration)
    - Department of Medical Science Mathematics — Professor Tatsuhiko Tsunoda
    - Frontier Research Unit Laboratory of Gene Expression — (under consideration)
- Joint Research Departments
  - Department of Precision Health
- Division of Integrative Research
  - Systems Biology for Intractable Diseases — Visiting Professors Seiya Imoto
- Intractable Disease Integrated Research Laboratory
- COVID-19 Integrated Research Laboratory
- Advanced Technology Laboratory
  - Genome Laboratory
  - Laboratory of Cytometry and Proteome Research
  - Laboratory of Genome Editing for Biomedical Research
  - Laboratory of Anatomy and Cell Function
  - Bioresource Laboratory
  - Laboratory of Structural Biology
  - Stem Cell Laboratory
- Administrative Office
  - General Affairs Section

Medical Research Institute (2022～)

- Director: Hiroshi Nishina
- Deputy Director
- Faculty Meeting
- 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 Molecular Epidemiology — (under consideration)
    - Department of Stem Cell Biology — (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 Molecular Neuroscience — Professor — Kohichi Tanaka
    - Department of Pathological Cell Biology — Professor — Shigeomi Shimizu
    - Department of Immunology — (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 Bio-informational Pharmacology — Professor — Tetsushi Furukawa
    - Department of Molecular Cytogenetics — (under consideration)
    - Department of Medical Science Mathematics — (under consideration)
    - Department of Epigenetics — (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
  - 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
    - Laboratory for Structure Analysis
    - Stem Cell Laboratory
    - Single-cell Omics Laboratoy
  - Administration Office
    - General Affairs Unit

Inter-University Research Network for Trans-Omics Medicine

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



Aim of the Project

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

Participating Joint Usage/Research Centers

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

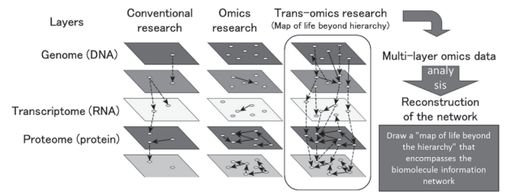
Activities

* **Joint research symposium**

The 6th Symposium of the Inter-University Research Network for Trans-Omics Medicine
New Technologies Meet Biology
Date: January 18-19, 2021
Place: Online symposium at ZOOM

In order to truly understand biological phenomena and disease mechanisms, it is necessary to reconstruct the information network we woven from multiple hierarchical omics data to understand cell strategies (trans-omics research). However, the protocol of trans-omics research does not exist, there are no human resources to realize nor the foundation (platform). Therefore, in this project, we will develop the world's first common protocol of trans-omics research ("New map of life"), establish research platform and human resource development.

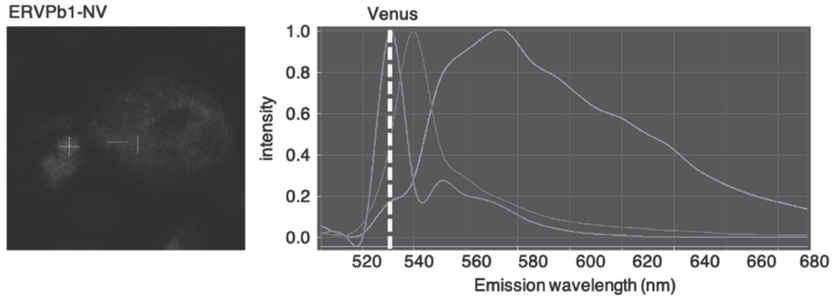
In this project, Medical Research Institute (for intractable disease research) acquires omics data mainly on three layers of genomics, epigenomics and transcriptomics. Promote creative research that can be a model of trans-omics research by systematically conducting research through cooperation with other three centers.



Identification of Retrovirus-Derived Genes Using a Method for Detecting the In Vivo Expression Sites of Lowly Expressed Proteins

Antibody detection methods are routinely used for protein expression analysis, but antibody specificity to targets is sometimes insufficient. Even newly developed "Ribosome profiling", a technique that identifies ORFs by sequencing of their ribosome-binding site, may overlook transient low-expression proteins. Therefore, we have developed a novel method to introduce fluorescent proteins into target genes using the CRISPR/Cas9 system. We expect that this system enables not only to detect many novel proteins that have not been analyzed due to their low expression levels but also to determine their localization and temporal expression by detecting fluorescence from Venus or mCherry fused with the target proteins. Actually, we confirmed that it is possible to detect a very low amount of protein, such as the ERVPb1 protein encoded in one of retroviral envelop genes. *ERVPb1* is a Simiiformes-specific gene, and its expression occurred transiently in the differentiation system into macrophages, therefore, demonstrating that it is actually translated into protein. The expression of *ERVPb1* is upregulated by LPS stimulation, suggesting that it may play a role in innate immune system.

Matsuzawa A. *et al.* HERV-Derived Ervpb1 Is Conserved in Simiiformes, Exhibiting Expression in Hematopoietic Cell Lineages Including Macrophages. Int J Mol Sci. (2021) 22(9):4504,



Division of Advanced Molecular Medicine

Aim and Scope

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

【Medical Chemistry】

- Identification of a point mutation in phospholipid flippase in patients with psychosomatic disorders and elucidation of their pathological mechanisms
- Identification of a phospholipid scramblase regulated by ATP receptor P2X7

【Molecular Cell Biology】

- SOX2 expression is required for CSCs generation by oncogenic RAS mutation.
- RAS/RAF/MAPK pathway-induced CDK1 activation is important for induction of *O*-GlcNAcylation.

【Molecular Neuroscience】

- Activation of CaMKII-CREB pathway protects retinal ganglion cells from excitotoxicity.
- Nprl2- and Nprl3-conditional knockout mice recapitulated the abnormal features of patients with focal epilepsies
- Mice with reduced glutamate transporter GLT1 expression exhibit behaviors related to attention-deficit/hyperactivity disorder

【Biodefense Research】

- Using an antibody-drug conjugate (ADC) that targets human monocyte progenitors (cMoPs), we developed the basis of new cancer treatments.
- We established unique organoid libraries of human tongue cancer and esophageal cancer.

【Bio-informational Pharmacology】

- We identified cfDNA as a potential novel biomarker for atrial fibrillation, and demonstrated that cfDNA contributes to the induction of systemic inflammation in atrial fibrillation.
- Elucidation of the common mechanism of congenital heart disease development and tumorigenesis by Tbx5-signaling.

【Stem Cell Regulation】

- Development of a synthetic polymer that maintains neural stem cells in vitro in serum-free medium without growth factors
- Identification of Rasip1 that functions in the maintenance of hematopoietic ability of intra-aortic hematopoietic cell clusters in midgestation mouse dorsal aorta
- Discovery of a self-expanding strategy of glioma stem cells (GSCs) that systemically exploit erythroid lineage cells

【Structural Biology】

- Hemoglobin shows two district structures with/without oxygen molecules bound on it. We have determined a series of intermediate structures of a hemoglobin to understand the detail of cooperativity, which is characteristic of the protein.
- Ultra-high resolution structure of an oxidoreductase was determined by both X-ray and neutron diffractions to elucidate the find detail of the chemical reaction catalyzed by it.

Department of Medical Chemistry

Professor Katsumori Segawa, Ph.D.
Assistant Professor Yugo Miyata, Ph.D.

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 leaflets of the plasma membrane. We aim to identify various phospholipid translocases and elucidate their physiological and pathophysiological significances.

1. Background

Flippases establish and maintain asymmetrical distribution of PtdSer and PtdEtn by flipping them in one direction from the outer to the inner leaflet of the lipid bilayer. We found that ATP8A2, ATP11A, and ATP11C, which belong to the type IV P-type ATPase (P4-ATPase) family, are flippases that function at the plasma membrane. These members form a stable flippase complex with CDC50A, an essential subunit for the family, and localize at the plasma membrane to flip 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. An analysis of flippase activity using cell-based assay and purified flippase complexes in vitro showed that the three members have comparable PtdSer flippase activity. Indeed, T lymphoid cells doubly deficient in ATP11A and ATP11C lose plasma membrane flippase activity and continue to expose 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.

2. Identification of a sublethal ATP11A mutation associated with neurological deterioration

Phospholipid regulation mediated by flippases is physiologically significant. Mice deficient in ATP11C exhibit B-cell lymphopenia, cholestasis, anemia, and dystocia, and point mutations in the human *ATP11C* gene also cause anemia. In addition, *ATP11A*-deficient mice are embryonically lethal due to the poor development of syncytiotrophoblasts in the placenta. Recently, Dr. Kure and his colleagues at Tohoku University found a patient with a point mutation in the *ATP11A* gene. The patient developed epilepsy after birth and showed neurological deterioration with cerebral atrophy and leukodystrophy from infancy. Whole-exome sequencing analysis between healthy parents and the patient identified a heterozygous point mutation in the *ATP11A* gene (c.250C>G, p.Q84E). Indeed, more than 80% of the heterozygous knock-in mice carrying this point mutation were lethal at one week of age, and the surviving mice showed signs of neurological deterioration. These results indicated that the Q84E mutation caused the symptoms in the patient. The Q84 of human ATP11A corresponded to Q79 of ATP11C, which formed a salt bridge with a head group of PtdSer, suggesting that the flippase activity or substrate specificity toward PtdSer may be affected by the mutation. Therefore, we expressed the ATP11A-Q84E mutant in cells and examined flippase activity on the plasma membrane. We found that the Q84E mutant flipped PtdCho without losing its activity toward PtdSer and PtdEtn. We then performed molecular dynamics simulations to understand molecular mechanisms for the PtdCho flipping by the Q84E mutant and found that the binding energy between PtdCho and the mutated glutamic acid was increased by newly formed electrostatic interactions, which facilitates retention of PtdCho in the lipid entry cavity of ATP11A. Next, we performed a microarray analysis using the T-lymphoma cell line expressing the Q84E mutant and found that the expression of sphingomyelin synthase 1 (SGMS1) was increased by approximately 20-fold. Mass spectrometry analysis revealed a 2-fold increase in SM in cells expressing the Q84E mutant. Accordingly, a marked decrease in PtdCho and an increase in SM in the outer leaflet of the plasma membrane was observed in the mutant-expressing cells. This lipid re-organization in the plasma membrane caused several deteriorations in cell integrity, such as decreased proliferative capacity, decreased cell size, and cell rupture by extracellular sphingomyelinase. Finally, using imaging mass spectrometry, we confirmed that SM is increased in fetuses of Q84E heterozygous knock-in mice. These results indicate that the composition ratio of PC to SM in the outer leaflet of the plasma membrane is essential to maintain cellular homeostasis and survival (Figure. 1).

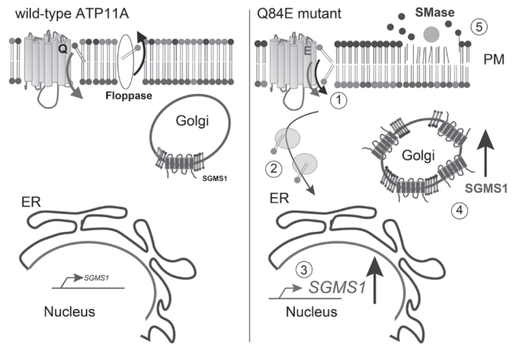


Figure 1 Schematic model of the cells expressing the Q84E mutant of ATP11A. Left panel: In normal cells, ATP11A translocates PtdSer and PtdEtn from the outer to the inner leaflets of the plasma membranes, while a putative floppase(s) translocates PtdCho from the inner to the outer leaflets, thus maintaining most of PtdSer and PtdEtn in the inner leaflet and PtdCho in the outer leaflet. Right panel: (1) The Q84E mutant ATP11A translocates not only PtdSer and PtdEtn, but also PtdCho. PtdCho is moved to the inner leaflet of the plasma membrane, and some of it is transferred to the ER (2). The ER seems to have a mechanism to sense the increase in PtdCho concentration to up-regulate the SGMS1 gene expression (3). The SGMS1 in the Golgi synthesises SM from PtdCho and ceramide, and the resultant SM moves to the plasma membrane (4). The high concentration of SM with the reduced concentration of PtdCho sensitises the cells against sphingomyelinase for cell rupture (5).

3. Identification of a phospholipid scramblase responsible for the P2X7-meidated phospholipid scrambling

Extracellular ATP levels are increased by cell death associated with tissue damage and inflammation, activating immune cells such as macrophages and regulatory T cells via an ATP receptor, P2X7. In particular, regulatory T cells exposed to high concentrations of ATP rapidly undergo cell death while exposing PtdSer. In a collaborative study with Dr. Nagata, we performed a genome-wide CRISPR/Cas9 screen to identify molecules responsible for the phospholipid scrambling in the plasma membrane of T cells activated by extracellular ATP and identified Xk and Vps13a. Xk is a paralog of a scramblase, Xkr8, and Vps13a is a cytosolic protein with lipid transport activity. Indeed, the P2X7-dependent scrambling activity was compromised in T cell lines deficient in Xk or Vps13a. The loss of Xk or Vps13a also suppressed cell death triggered by high levels of extracellular ATP. Biochemical analysis and confocal microscopy showed that Xk and Vps13a formed a complex and localized to the plasma membrane. From these results, we concluded that the Xk-Vps13a complex present in the plasma membrane was responsible for phospholipid scrambling induced by P2X7. Patients with McLeod syndrome and chorea-acanthocytosis carry the mutation in *XK* and *VPS13A* genes, respectively, suggesting that a loss of phospholipid scrambling by the Xk-Vps13a complex at the plasma membrane causes these diseases.

Annual publications

1. A sublethal ATP11A mutation associated with neurological deterioration causes aberrant phosphatidylcholine flipping in plasma membranes.
Segawa K, Kikuchi A, Noji T, Sugiura Y, Hiraga K, Suzuki C, Haginoya K, Kobayashi Y, Matsunaga M, Ochiai Y, Yamada K, Nishimura T, Iwasawa S, Shoji W, Sugihara F, Nishino K, Kosako H, Ikawa M, Uchiyama Y, Suematsu M, Ishikita H, Kure S, Nagata S.
J Clin Invest. 131(18):148005, 2021.

2. Sensing and clearance of apoptotic cells.
Nagata S, Segawa K.
Curr Opin Immunol. 68:1-8, 2021.

3. Requirement of Xk and Vps13a for the P2X7-mediated phospholipid scrambling and cell lysis in mouse T cells.
Ryoden Y, Segawa K, Nagata S.
Proc Natl Acad Sci U S A. in press.

Department of Molecular Cell Biology

Professor **Hiroshi Shibuya**
Associate Professor **Toshiyasu Goto**
Assistant Professor **Masahiro Shimizu**

Overview

Cancer stem cells (CSCs), also called tumor-initiating cells, are a subset of tumor cells that exhibit self-renewal ability and generate the diverse cells that comprise the tumor. CSCs show increased quiescence and poor responses to conventional chemotherapy strategies that primarily kill proliferating cells. Therefore, CSCs are correlated with chemoresistance, invasion and relapse of cancer cells. However, the underlying mechanisms of CSCs generation have not been completely elucidated.

Introduction

Alterations of genes involved in the regulation of cell proliferation, such as oncogenes and tumor suppressor genes, may lead to cancer development. Therefore, it is possible that analyzes of reprogramming factors and cell growth regulators will lead to the elucidation of the mechanism of CSCs generation. However, cancer develops because of the acquisition of successive hallmark cancer capabilities in a multistep pathogenic process, and it is difficult to analyze the role of reprogramming factors in CSCs generation using established cancer cell lines. To solve these problems, we use normal cells expressing oncogenic mutants.

1. Oncogenic RAS induces SOX2-initiated CSCs generation.

Previously, we reported that oncogenic HRAS (HRAS G12V mutant: $HRAS^{V12}$) induced tumorigenic properties in p53-deficient mouse embryonic fibroblasts ($p53^{-/-}$MEFs), suggesting that $HRAS^{V12}$ promoted the generation of CSCs. To confirm this hypothesis, we generated $HRAS^{V12}$-expressing $p53^{-/-}$MEFs and demonstrated that these cells formed tumors in nude mice. It has been reported that cancer cells with CSC-like properties will form spheres in low attachment culture conditions in medium containing growth factors. We next examined CSCs development of $HRAS^{V12}$-expressing $p53^{-/-}$MEFs using sphere formation analysis, and found that approximately 0.4% of these cells developed spheres in low attachment plates. This demonstrates oncogenic RAS mutation generates CSCs. We also compared expression levels of reprograming factors such as OCT4, SOX2 and KLF4 between adherent $HRAS^{V12}$-expressing $p53^{-/-}$MEFs cells and $p53^{-/-}$MEFs control cells. Interestingly, the expression of only SOX2 was markedly enhanced in $HRAS^{V12}$-expressing $p53^{-/-}$MEFs cells, suggesting that it was possible that SOX2 functioned as an initiation factor for CSCs reprogramming. To test this hypothesis, we deleted the *SOX2* gene in $HRAS^{V12}$-expressing $p53^{-/-}$MEFs with three independent gRNAs using the CRISPR-Cas9 gene knockout system. We analysed the effect of *SOX2* KO on the CSC properties found in $HRAS^{V12}$-expressing $p53^{-/-}$MEFs, and showed that sphere-forming and tumor-initiating activities were not observed after *SOX2* KO. These results suggest that SOX2 expression is required for CSCs generation by oncogenic RAS mutation.

2. SOX2 expression is induced by the RAF-MAPK-CDK1 pathway in $HRAS^{V12}$-expressing $p53^{-/-}$MEFs.

RAS proteins directly activate the downstream effectors RAF and PI3K followed by the downstream activation of MAPK and AKT pathways, respectively. Therefore, we next examined whether these effectors were involved in promoting SOX2 expression. Constitutively active forms of RAF ($BRAF^{V600E}$) and PI3K ($PI3K^{CAAX}$) were stably expressed in $p53^{-/-}$MEFs. Although the increase in *SOX2* mRNA expression was relatively weakly in $PI3K^{CAAX}$-expressing cells, the levels of *SOX2* and *KLF4* mRNA in $BRAF^{V600E}$-expressing cells were similar to that in $HRAS^{V12}$-expressing cells. Furthermore, SOX2 expression was significantly reduced by the MEK inhibitors U0126 and PD184352 but not by the PI3K inhibitor LY294002 in $HRAS^{V12}$-expressing $p53^{-/-}$MEFs. These results suggest that the RAF/MEK/ERK pathway is required for SOX2 induction.

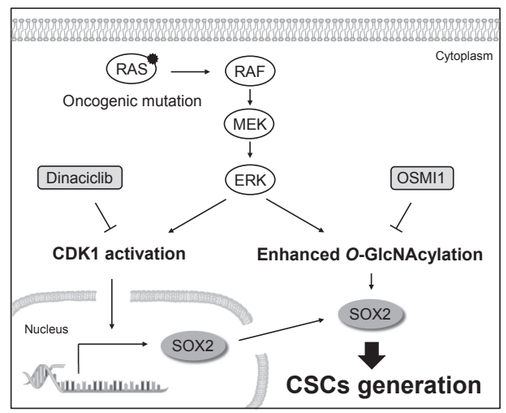
Activation of ERK enhances expression of cyclin D1, which binds to and activates cyclin-dependent kinase 4 and 6 (CDK4/6) during the G1 phase of the cell cycle. In addition to CDK4/6, the BRAF/MEK/MAPK pathway is also essential for some functions of CDK1 and CDK2. We next examined the effect of CDK4/6 inhibitor palbociclib and CDK1/2 inhibitor dinaciclib on SOX2 expression. As a result, although the expression of *SOX2* mRNA and protein levels were slightly reduced in $HRAS^{V12}$-expressing $p53^{-/-}$MEFs after treatment with a high-dose of palbociclib, *SOX2* mRNA and protein expression was strongly suppressed by dinaciclib. Moreover, the number of sphere-forming cells in $HRAS^{V12}$-expressing $p53^{-/-}$MEFs was largely suppressed by dinaciclib but only partially suppressed by palbociclib. To further analyse whether CDKs were essential for SOX2 expression, we analysed SOX2 levels in $HRAS^{V12}$-expressing $p53^{-/-}$MEFs after knockdown of *CDK1* or *CDK2* using siRNA, and confirmed requirement of only CDK1 for SOX2 induction. These results indicate that CDK1 activity is required for SOX2 expression in $HRAS^{V12}$-expressing $p53^{-/-}$MEFs.

3. Enhanced *O*-GlcNAc modification induced by the RAS/MAPK/CDK1 pathway is required for SOX2 protein expression and generation of cancer stem cells.

GlcNAcylation is the post-translational modification of N-acetylglucosamine (also known as GlcNAc) to serine or threonine residues of proteins that contributes to stability and activity of modified proteins. Previously, we reported that enhanced *O*-GlcNAcylation is important for SOX2 expression and maintenance of CSCs properties, including sphere- and tumour-forming activities, in colon and lung cancer cells. These findings suggested the possibility that *O*-GlcNAc modifications are involved in acquisition of CSCs properties. Therefore, we determined the *O*-GlcNAc levels in $HRAS^{V12}$-expressing $p53^{-/-}$MEFs and found elevated levels of protein *O*-GlcNAcylation compared with control $p53^{-/-}$MEFs. Next, we analyzed the cells after treatment with OSMI1, a cell-permeable, small molecule *O*-GlcNAc transferase inhibitor, and found that OSMI1 treatment suppressed total *O*-GlcNAcylation levels of proteins and SOX2 expression in $HRAS^{V12}$-expressing $p53^{-/-}$MEFs. Interestingly, the OSMI1-mediated reduction in SOX2 levels was attenuated by treatment with the proteasome inhibitor MG-132 and the mRNA levels of *SOX2* in $HRAS^{V12}$expressing $p53^{-/-}$MEFs were not significantly changed by OSMI1, indicating that *O*-GlcNAcylation regulated SOX2 expression at the post-transcriptional level. Consistent with these results, the numbers of sphere-forming cells decreased after treatment with OSMI1. These results suggest that increased *O*-GlcNAcylation is required for SOX2 protein expression and sphere-forming activity in these cells.

Finally, we analyzed the role of RAS/MAPK-activated CDK1 in the induction of *O*-GlcNAcylation. The elevated levels of *O*-GlcNAcylation in $HRAS^{V12}$-expressing $p53^{-/-}$MEFs were suppressed by dinaciclib in a dose dependent manner. Furthermore, knockdown of *CDK1*, but not *CDK2*, with siRNA inhibited *O*-GlcNAcylation levels in the cells. In addition, SOX2 expression and *O*-GlcNAcylation levels in KRAS-activated cancer cells including colon cancer cells HCT116, SW480 and DLD1 and lung cancer cells H460 and A549 were suppressed by dinaciclib treatment.

In conclusion, we show that RAS/RAF/MAPK pathway-induced CDK1 activation is important for induction of *O*-GlcNAcylation, and this activation pathway is required for SOX2 expression and subsequent CSC generation. Our results suggest the possibility that this signaling pathway is a therapeutic target for CSCs.



Mechanism of CSCs generation by oncogenic signal

Publications

Shimizu M, Shibuya H, Tanaka N. Enhanced *O*-GlcNAc modification induced by the RAS/MAPK/CDK1 pathway is required for SOX2 protein expression and generation of cancer stem cells. ***Scientific Reports*** 12, Article number: 2910 (2022).

Department of Molecular Neuroscience

Professor Kohichi Tanaka
Assistant Professor Yuichi Hiraoka
Assistant Professor Tetsuo Ohnishi

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

1. Functions of glutamate transporters in the brain

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

Attention-deficit/hyperactivity disorder (ADHD) is a common neuropsychiatric disorder in children. Although animal models and human brain imaging studies indicate a significant role for glutamatergic dysfunction in ADHD, there is no direct evidence that glutamatergic dysfunction is sufficient to induce ADHD-like symptoms. The glial glutamate transporter GLT1 plays a critical role in glutamatergic neurotransmission. We report the generation of mice expressing only 20% of normal levels of the GLT1. Unlike conventional GLT1 knockout mice, these mice survive to adulthood and exhibit ADHD-like phenotypes, including hyperactivity, impulsivity and impaired memory. These findings indicate that glutamatergic dysfunction due to GLT1 deficiency, a mechanism distinct from the dopaminergic deficit hypothesis of ADHD, underlies ADHD-like symptoms.

2. Novel mouse models for focal epilepsy

The most frequent genetic cause of focal epilepsies is variations in the GAP activity toward RAGs 1 complex genes DEP domain containing 5 (DEPDC5), nitrogen permease regulator 2-like protein (NPRL2) and nitrogen permease regulator 3-like protein (NPRL3). Because these variations are frequent and associated with a broad spectrum of focal epilepsies, a unique pathology categorized as GATORopathy can be conceptualized. Animal models recapitulating the clinical features of patients are essential to decipher GATORopathy. Although several genetically modified animal models recapitulate DEPDC5-related epilepsy, no models have been reported for NPRL2- or NPRL3-related epilepsies. Here, we conditionally deleted Nprl2 and Nprl3 from the dorsal telencephalon in mice [Emx1cre/+; Nprl2f/f (Nprl2-cKO) and Emx1cre/+; Nprl3f/f (Nprl3-cKO)] and compared their phenotypes with Nprl2+/-, Nprl3+/- and Emx1cre/+; Depdc5f/f (Depdc5-cKO) mice. Nprl2-cKO and Nprl3-cKO mice recapitulated the major abnormal features of patients-spontaneous seizures, and dysmorphic enlarged neuronal cells with increased mechanistic target of rapamycin complex 1 signaling-similar to Depdc5-cKO mice. Chronic postnatal rapamycin administration dramatically prolonged the survival period and inhibited seizure occurrence but not enlarged neuronal cells in Nprl2-cKO and Nprl3-cKO mice. Further studies using these conditional knockout mice will be useful for understanding GATORopathy and for the identification of novel therapeutic targets.

3. Analysis of a novel gene expression regulatory system related to the pathophysiology of mental disorders

Patients with schizophrenia, one of severe mental disorders, suffer from a variety of symptoms including hallucination, delusion, affective flattening and cognitive deficits. Although the genetic predisposition contributes to the pathogenesis of this illness, its genetic architecture remains still to be elucidated. We have reported on a schizophrenia patient who harbored a balanced chromosomal translocation, and identified the gene *LDB2* (Lim Domain-Binding 2), which locates proximately to the chromosomal breakpoint. *Ldb2* KO mice exhibited multiple deficits related to schizophrenia, strongly supporting that *LDB2* is a causal gene in the proband patient. On a basis of the fact that LDB2 is a potential transcription regulator, we conducted ChIP-seq analyses with an LDB2-specific antibody. Of interest, we found that LDB2 binds to ~10,000 genomic sites in human neurosphreres, and that its DNA binding is indirectly mediated by the EGR (early growth response) transcription factors, which are implicated in the pathophysiology of schizophrenia. Taken together, we proposed that a novel gene expression regulatory system, which we named the 'LDB2-EGR axis', regulates synapse dynamics via controlling expressions of downstream genes.

Publications

[Original papers]

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8. Balan, S., Ohnishi, T., Watanabe, A., Ohba, H., Iwayama, Y., Toyoshima, M., Hara, T., Hisano, Y., Miyasaka, Y., Toyota, T., Shimamoto-Mitsuyama, C., Maekawa, M., Numata, S., Ohmori, T., Shimogori, T., Kikkawa, Y., Hayashi, T., Yoshikawa, T. Role of an atypical cadherin gene, Cdh23 in prepulse inhibition, and implication of CDH23 in Schizophrenia. *Schizophr. Bull* 47. 1190-1200, 2021.
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Department of Biodefense Research

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Adjunct Lecturer	Nobuyuki Onai, Ph.D.
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Research Technician	Toyoki Hayashi
Secretarial Assistant	Hisako Kamioka

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

1. Research on myeloid cells

1) Identification of novel sources of dendritic cells and macrophages

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 common DC progenitors (CDPs) (***Immunity*** 2013; ***Nat Immunol*** 2007). Interestingly, we found that CDPs are divided into 2 subpopulations. One is M-CSF receptor (R)$^+$ CDPs mainly producing cDCs, and the other M-CSFR$^-$CDPs producing a large number of pDCs.

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 (**Fig. 1**).

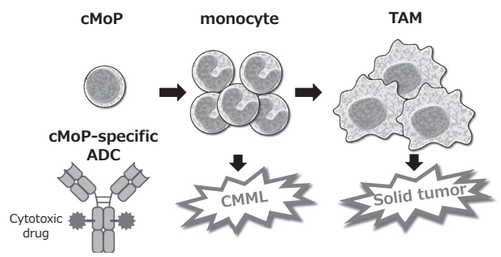

Fig.1 A new cancer treatment basis targeting cMoP

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

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

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

2. Research on tissue stem cells

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

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

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

2) Establishment of human tongue cancer organoid biobank

Squamous cell carcinoma occurs in the oral cavity, esophagus, lungs, and cervix. Tongue cancer accounts for about 60% of oral cancer, and the 5-year survival rate is extremely low for advanced tongue cancer, and the recurrence rate after radical treatment is also high. Similarly, squamous cell carcinoma of the esophagus, which is characteristic of Asian countries including Japan, has a very high recurrence rate after curative treatment. As a multicenter collaborative study, our research group has succeeded in constructing an organoid library specialized for human tongue cancer and human esophageal squamous cell carcinoma, which has never been reported (34 cases of tongue cancer and 18 cases of esophageal cancer, ongoing) (**Fig. 2**). 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 are in progress.

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2. Izumi Y, Kanayama M, Shen Z, Kai M, Kawamura S, Akiyama M, Yamamoto M, Nagao T, Okada K, Kawamata N, Toyota S, Ohteki T. An antibody-drug conjugate that selectively targets human monocyte progenitors for anti-cancer therapy. **Front Immunol** 12, 618081 (2021). Doi: 10.3389/fimmu.2021.618081.

Personnel Changes

Moving out:

Kajita M, Assistant Professor of Ritsumeikan University (April 1, 2021).

Moving in:

Sato H, General Exchange Special Research Student from Jichi Medical University (April 1, 2021).

Ohashi E and Nakagawa M, Master's Program of Tokyo Medical and Dental University (April 1, 2021).

Department of Bio-informational Pharmacology

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

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

1. cfDNA and atrial fibrillation

Atrial fibrillation (AF) is one of the most common arrhythmias encountered in clinical practice and is associated with severe cerebral infarction resulting in a major social burden. Although it is well known that inflammation is involved in the AF pathogenesis, the mechanism by which AF induces inflammation has not been elucidated, while many reports indicate that inflammation can cause AF.

We have researched on cell-free DNA (cfDNA) in the blood to investigate the relationship between cfDNA and AF (Yamazoe M, et al. Sci Rep 2021). cfDNA has been well studied in the field of oncology, however its significance in AF is unclear. We quantified cfDNA from peripheral blood in patients with AF and found that the amount of cfDNA was higher in the elderly than in the young, in patients with AF than in healthy subjects, and in patients with persistent AF than in patients with paroxysmal AF. We also compared the copy numbers of nuclear-cfDNA (n-cfDNA) and mitochondrial-cfDNA (mt-cfDNA) and found that the copy number of mt-cfDNA correlated more with the presence of AF than that of n-cfDNA, suggesting that cfDNA, especially mt-cfDNA, may be useful as a biomarker for detecting AF. Furthermore, we found that the pacing of cultured cardiomyocytes in vitro increased cfDNA in the culture media and that the administration of the cfDNA to macrophages increased the expression of IL-1β and IL-6. In particular, this increase in cytokine expression was significantly induced by mt-cfDNA but not by n-cfDNA, suggesting that unmethylated mt-cfDNA induces a TLR-9 mediated pre-immune response and that AF can induce systemic inflammation via cfDNA.

2. Elucidation of the common mechanism of congenital heart disease development and tumorigenesis by Tbx5-signaling

We are focusing on the *Tbx5* gene, which is the responsible gene for human Holt-Oram syndrome, and its mutations cause upper limb defects and cardiac defects. By focusing on the transcriptional environment of Tbx5, our group aims to understand the mechanism of congenital heart disease and the mechanism of cardiac fate determination. In this year, we have demonstrated that one of Tbx5 targeting genes, glypican-5 (GPC5) by transcriptome analysis from *Tbx5* deficient hearts. GPC5 functions as a captive receptor that constantly transmits Shh-FGF signaling into cells. This result provides a strong insight into why the rate of atrial and ventricular septal defects is so high in human heart disease, which has not been elucidated. In addition, the authors performed this study using tumorigenic cells in which Tbx5 enhancement was observed. We also found that excessive GPC5 expression during undifferentiated tumor progenitor cells increases cell proliferation and promotes tumorigenesis (Takeuchi et al., ***PLOS ONE*** 2021). In the future, it is expected to clarify the mechanism by which GPC5 functions as a tumorigenic switch in tumorigenic stem cells and the mechanism by which GPC5, which appears to be localized during myocardial proliferation, is suppressed as myocardium matures.

Publications

[original articles]

1. Yamazoe M, Sasano T, Ihara K, Takahashi T, Nakamura W, Takahashi N, Komuro H, Hamada S, Furukawa T. Sparsely Methylated Mitochondrial Cell free DNA Released from Cardiomyocytes Contributes to Systemic Inflammatory Response Accompanied by Atrial Fibrillation. Sci Rep. 2021. 11: 5837. https://doi.org/10.1038/s41598-021-85204-7
2. Takeuchi M., Takeuchi K., Monobe Y., Takai T., Yamaguchi R., Furukawa T., Akagi K., Takeuchi JK*. Subcellular localization of glypican-5 is associated with dynamic motility of the human mesenchymal stem cell line U3DT. ***PLOS ONE*** doi.org/10.1371/journal.pone.0226538 2021 *: correspondence to this work

Department of Stem Cell Regulation

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Research Student	Zengye ZONGPEI

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 2021 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. Elucidation of molecular mechanisms underlying maintenance of neural stem cell self-renewal by developing niche-mimicking synthetic polymers

Neural stem cell (NSC) self-renewal is essential for the development, homeostasis, and regeneration of the central nervous system and is tightly regulated by multiple signals from their microenvironment, the niche. However, the neural stem cell niche has not been fully elucidated due to the complex interplay of biological, chemical, and physicochemical factors. Previously, we have established a unique high-throughput screening system based on "polymer microarrays" with arrays fabricated from a wide variety of synthetic polymers to identify polymer scaffolds that mimic the stem cell niche. In this study, we screened 376 acrylate, acrylamide, and urethane polymers, and identified the acrylate polymer PA518 as an NSC niche-mimetic polymer scaffold that maintains NSC self-renewal and pluripotency in serum-free medium without growth factors. NSCs cultured on PA518-coated dishes without serum and growth factors maintained (1) the expression of nestin, an NSC marker, (2) the BrdU labeling index, representing proliferative activity, and (3) the ability to form neurospheres, an indication of the self-renewal ability of NSCs (Figure 1). In addition, SDS-PAGE and silver staining suggested the presence of neural stem cell-derived proteins that bind to PA518. These results indicate that PA518 is a useful polymer for a comprehensive understanding of the neural stem cell niche, and further characterization of PA518-bound proteins is expected to provide new insights into the regulation of NSCs.

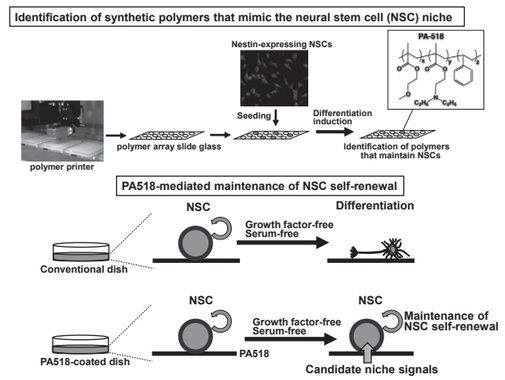

Figure 1: Identification of a niche mimicking synthetic polymer scaffold that maintains neural stem cells

2. Involvement of Rasip1 in the maintenance of hematopoietic ability of intra-aortic hematopoietic cell clusters in midgestation mouse dorsal aorta

During mouse embryonic development, hematopoietic stem cells (HSCs) firstly arise in intra-aortic hematopoietic cell clusters (IAHCs) of the dorsal aorta at midgestation. We have previously reported that forced expression of a transcription factor Sox17, which is expressed in IAHCs, into IAHC cells maintains the formation of hematopoietic cell cluster with the hematopoietic activity, while knockdown of Sox17 in Sox17-transduced cells leads to a decrease in the cluster-forming activity and the hematopoietic activity. Moreover, we revealed that Sox17-induced gene expression of Notch1 and adhesion molecules is important to maintain the undifferentiated state and the cluster formation. However, the role of Sox17 in hematopoiesis has not fully been explained. In the present study, we search a novel Sox17 downstream target involved in the maintenance of HSCs. First, we examined genes with high expression in Sox17-expressing IAHC cells in knock-in mice expressing a GFP gene under the control of the Sox17 promoter. We focus on Ras interacting protein 1 (Rasip1), which is a vascular-specific regulator of GTPase signaling, cell architecture, and adhesion. Rasip1 is expressed under the membrane of IAHCs in E10.5 embryos. Rasip1 is highly expressed in Sox17-transduced cells derived from IAHC cells n midgestational mouse embryos. Overexpression of Rasip1 in IAHC cells led to high hematopoietic activity, while knockdown of Rasip1 in Sox17-transduced cells impeded the cluster formation and diminished the hematopoietic ability. The reduced luciferase activity was observed when the Sox17-binding sequence in the putative Rasip1 promoter region was mutated. These results strongly suggest that the functional role of Rasip1 in maintenance of hematopoietic activity of HSC-containing IAHCs is requisite (Figure 2).

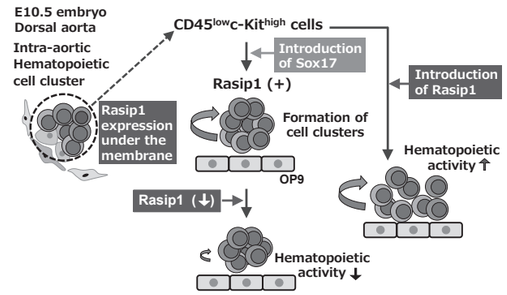

Figure 2: Model of the role of Sox17-induced Rasip1 expression in the hematopoietic ability of intra-aortic hematopoietic cell clusters

3. A self-expanding strategy of glioma stem cells (GSCs) that systemically exploit erythroid lineage cells

"Cancer stem cells" (CSCs), a small subset of tumor cells, are characterized by chemo/radio-resistance and have the ability to reconstitute original tumors. Therefore, CSCs are a key driver of tumor relapse and have been proposed as a promising target to eradicate cancers (Figure 3). Previously, we have demonstrated that glioma stem cells (GSCs) recruit and induce the differentiation of bone marrow (BM) monocytes into tumor-infiltrating macrophages, which phagocytose hemorrhaged erythrocytes and store GSC-beneficial iron in mouse xenografts, suggesting a self-expanding strategy of GSCs that exploits host hematopoiesis of myeloid cells. However, it remains unclear whether a self-advantageous effect of GSCs also occurs on erythroid cells during glioma development. Using the primary cultures of mouse fetal liver proerythroblasts (proEs), we found that conditioned media prepared from glioma cells including patient-derived glioblastoma (GBM) cells significantly facilitated the differentiation of proEs into erythroblasts. Importantly, in vivo erythroid analysis in intracranially GSC-transplanted mice showed an enhanced erythropoiesis in the BM. In addition, the sphere forming ability of patient-derived GBM cells was significantly suppressed by hypoxia treatment and iron chelation, suggesting higher demands of GSCs for oxygen and iron, which may be supplied by GSCs- and their progeny-induced erythrocyte production. This is the first demonstration that brain tumor remotely interacts with host erythropoiesis, underscoring the importance of studying glioma as a systemic disease, and suggest a new strategy of GSCs for self-expansion by systemically exploiting erythroid lineages to replenish oxygen and iron.

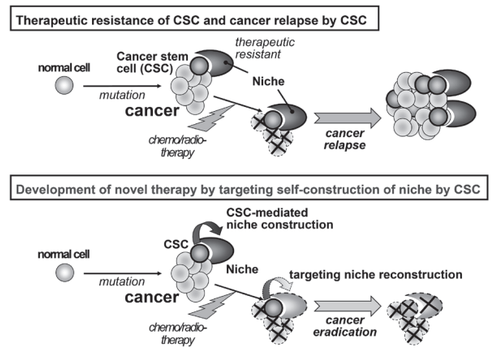

Figure 3: The concept of CSC-mediated niche construction and cancer eradication

Publications

[Original Article]

Suzuki I, Yoshida S, Tabu K, Kusunoki S, Matsumura Y, Izumi H, Asanoma K, Yagi H, Onoyama I, Sonoda K, Kohno K, Taga T, Takeda S, Kato K. YBX2 and cancer testis antigen 45 contribute to stemness, chemoresistance and a high degree of malignancy in human endometrial cancer Scientific Reports, 11(1); 4220, 2021

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. Structural basis for the allosteric intermediates of hemoglobin

The development of a practical blood substitute has not been successful even at present, and methods for increasing its molecular weight, such as a PEGylation or polymerization of hemoglobin (Hb) are being investigated to substitute for red blood cell. In addition, blood products for transfusion have a problem that autoxidation gradually occurs during storage and the oxygen carrying capacity of Hb is lost. However, Hbs with a huge molecular weight of several to tens of times that of human Hb occur in some invertebrates such as annelids (Fig. 1A). Some of these giant Hbs have the characteristic that they show stronger resistance to autoxidation than that of human Hb. Therefore, the detailed mechanism of cooperative oxygen binding/dissociation and the molecular mechanism of anti-autoxidation based on the three-dimensional structure of giant Hb are crucial information for the development of blood substitutes.

We have determined the crystal structures of the giant Hb in both oxygenated (oxy) and deoxygenated (deoxy) forms. In this process, we found that the crystals of the oxy form can be transferred to the deoxy form without disrupting crystals. By applying this property, we have successfully determined the crystal structures of oxygen dissociation intermediates of the giant Hb. The obtained structures provide the structural basis for the details of the molecular mechanism by which multiple oxygen binding sites in Hb work cooperatively to efficiently bind, and release oxygen. This is the first achievement in the long history of Hb research. The structures demonstrate that oxygen dissociation occurred with a strong correlation in the dimer structure formed by the adjacent subunits (Fig. 1B). Furthermore, it was also clarified that a large quaternary structural change over the entire molecule occurs when about half of the oxygen is dissociated, although the local structural change precedes it.

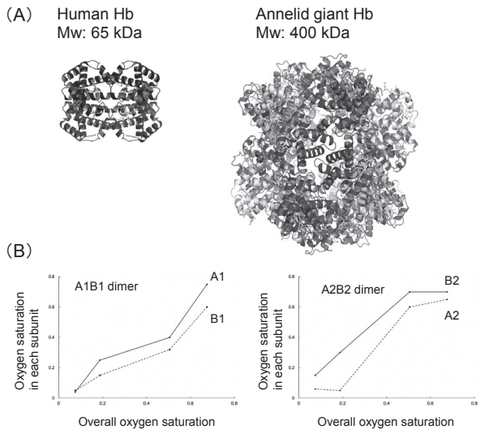

Fig.1 (A) Structures of human Hb and annelid giant Hb. (B) Transitions of oxygen saturation ratio of each subunit of the giant Hb.

This work is performed in collaboration with Kyoto University, Kanazawa University, RIKEN, and Japan Synchrotron Radiation Research Institute.

2. Ultra-high resolution structural analysis of the redox protein

To determine the location of hydrogen atoms in proteins, which account for roughly half of all atoms, is of great importance to clarify the molecular function of proteins. From the viewpoint of structure-based drug design, the information on hydrogen atoms is essential. However, it is difficult to determine the position of hydrogen atoms by X-ray crystallography or cryo-electron microscopy due to their low scattering power.

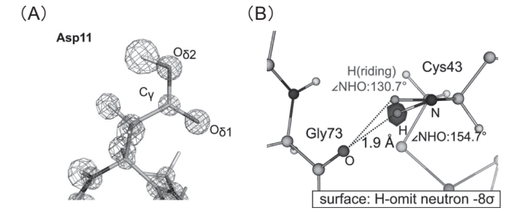

Fig.2 (A) Electron and nuclear density maps of HiPIP. (B) Deviations of hydrogen atoms from the peptide plane.

We collected X-ray and neutron diffraction data of oxidized High-potential iron-sulfur protein (HiPIP) at 0.66 Å and 1.2 Å resolution, respectively. The neutron diffraction data of oxidized HiPIP are high enough quality to determine the precise locations of the hydrogen nuclei (Fig. 2A). The bond lengths and angles for hydrogen atoms are generally constrained in the X-ray and neutron structural refinement as a riding model. However, the nuclear densities of the amide protons of oxidized HiPIP deviate from the peptide plane. Therefore, the coordinates of amide protons were refined without geometry restraints. In the case of single acceptor hydrogen bonds, most of the donor-hydrogen-acceptor refined angles are greater than those of the riding model. The nucleus positions of the amide protons shift toward the acceptor atoms (Fig. 2B). Moreover, the planarity of the H-N-C=O plane depends on the pyramidalization of the nitrogen atom.

This work is performed in collaboration with Kyoto University, National Institutes for Quantum Science and Technology, and Ibaraki University.

3. Improvement in Protein Data Bank

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

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Division of Pathophysiology

【Aim and Scope】

The Division of Pathophysiology aims to elucidate the basic mechanisms of biological phenomena and abnormalities in intractable diseases and develop new diagnostic and therapeutic methods for various diseases for which curative treatment is difficult. The research themes of each department are as follows;

【Neuropathology】

- DNA damage in embryonic neural stem cell determines FTLDs' fate via early-stage neuronal necrosis.
- Discovery of Hepta-Histidine which inhibits Tau Aggregation.
- Prediction and verification of the AD-FTLD common pathomechanism based on dynamic molecular network analysis
- HMGB1 signaling phosphorylates Ku70 and impairs DNA damage repair in Alzheimer's disease pathology
- Tau activates microglia via the PQBP1-cGAS-STING pathway to promote brain inflammation

【Biochemical Pathophysiology】

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

【Pathological Cell Biology】

- Development of alternative autophagy-inducing compound that protects neurodegenerative disorders in mice
- Elucidation of the important role of Nickel particles in Crohn's disease

【Developmental and Regenerative Biology】

- Discovery of the involvement of MKK7 in female mouse parental behavior
- Discovery of the involvement of choline metabolism in YAP-induced cell competition

【Stem Cell Biology】

- Discovery of the mechanisms for natural senolysis of epidermal stem cells

【Immunology】

- Elucidation of the mechanism that restores responsiveness of immunodeficient B lymphocytes during development.
- Development of highly immunogenic carriers for vaccines

Department of Neuropathology

Professor	Hitoshi Okazawa
Project Lecturer/Part-time Lecturer	Haruhisa Inoue, Masaki Sone
Junior Associate Professor	Kyota Fujita
Specially Appointed Junior Associate Professor	Hidenori Homma
Assistant Professor	Hikari Tanaka

[Outline]

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

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

[This year's progress]

1. DNA damage in embryonic neural stem cell determines FTLDs' fate via early-stage neuronal necrosis

The early-stage pathologies of frontotemporal lobal degeneration (FTLD) remain largely unknown. In VCP^{T262A}-KI mice carrying VCP gene mutation linked to FTLD, insufficient DNA damage repair in neural stem/progenitor cells (NSCs) activated DNA-PK and CDK1 that disabled MCM3 essential for the G1/S cell cycle transition. Abnormal neural exit produced neurons carrying over unrepaired DNA damage and induced early-stage transcriptional repression-induced atypical cell death (TRIAD) necrosis accompanied by the specific markers pSer46-MARCKS and YAP. In utero gene therapy expressing normal VCP or non-phosphorylated mutant MCM3 rescued DNA damage, neuronal necrosis, cognitive function, and TDP43 aggregation in adult neurons of VCP^{T262A}-KI mice, whereas similar therapy in adulthood was less effective. The similar early-stage neuronal necrosis was detected in $PGRN^{R504X}$-KI, $CHMP2B^{Q165X}$-KI, and TDP^{N267S}-KI mice, and blocked by embryonic treatment with AAV–non-phospho-MCM3. Moreover, YAP-dependent necrosis occurred in neurons of human FTLD patients, and consistently pSer46-MARCKS was increased in cerebrospinal fluid (CSF) and serum of these patients. Collectively, developmental stress followed by early-stage neuronal necrosis is a potential target for therapeutics and one of the earliest general biomarkers for FTLD.

2. Prediction and verification of the AD-FTLD common pathomechanism based on dynamic molecular network analysis

Multiple gene mutations cause familial frontotemporal lobar degeneration (FTLD) while no single gene mutations exists in sporadic FTLD. Various proteins aggregate in variable regions of the brain, leading to multiple pathological and clinical prototypes. The heterogeneity of FTLD could be one of the reasons preventing development of disease-modifying therapy. We newly develop a mathematical method to analyze chronological changes of PPI networks with sequential big data from comprehensive phosphoproteome of four FTLD knock-in (KI) mouse models ($PGRN^{R504X}$-KI, $TDP43^{N267S}$-KI, VCP^{T262A}-KI and $CHMP2B^{Q165X}$-KI mice) together with four transgenic mouse models of Alzheimer's disease (AD) and with $APP^{KM670/671NL}$-KI mice at multiple time points. The new method reveals the common core pathological network across FTLD and AD, which is shared by mouse models and human postmortem brains. Based on the prediction, we performed therapeutic intervention of the FTLD models, and confirmed amelioration of pathologies and symptoms of four FTLD mouse models by interruption of the core molecule HMGB1, verifying the new mathematical method to predict dynamic molecular networks.

3. HMGB1 signaling phosphorylates Ku70 and impairs DNA damage repair in Alzheimer's disease pathology

DNA damage is increased in Alzheimer's disease (AD), while the underlying mechanisms are unknown. Here, we employ comprehensive phosphoproteome analysis, and identify abnormal phosphorylation of 70 kDa subunit of Ku antigen (Ku70) at Ser77/78, which prevents Ku70- DNA interaction, in human AD postmortem brains. The abnormal phosphorylation inhibits accumulation of Ku70 to the foci of DNA double strand break (DSB), impairs DNA damage repair and eventually causes transcriptional repression-induced atypical cell death (TRIAD). Cells under TRIAD necrosis reveal senescence phenotypes. Extracellular high mobility group box 1 (HMGB1) protein, which is released from necrotic or hyper-activated neurons in AD, binds to toll-like receptor 4 (TLR4) of neighboring neurons, and activates protein kinase C alpha (PKC α) that executes Ku70 phosphorylation at Ser77/78. Administration of human monoclonal anti-HMGB1 antibody to post-symptomatic AD model mice decreases neuronal DSBs, suppresses secondary TRIAD necrosis of neurons, prevents escalation of neurodegeneration, and ameliorates cognitive symptoms. TRIAD shares multiple features with senescence. These results discover the HMGB1-Ku70 axis that accounts for the increase of neuronal DNA damage and secondary enhancement of TRIAD, the cell death phenotype of senescence, in AD.

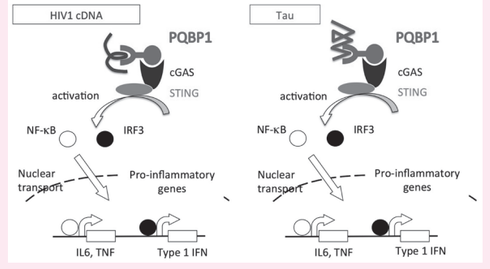
4. Tau activates microglia via the PQBP1-cGAS-STING pathway to promote brain inflammation

Brain inflammation generally accompanies and accelerates neurodegeneration. Here we report a microglial mechanism in which polyglutamine binding protein 1 (PQBP1) senses extrinsic tau 3R/4R proteins by direct interaction and triggers an innate immune response by activating a cyclic GMP-AMP synthase (cGAS)-Stimulator of interferon genes (STING) pathway. Tamoxifen-inducible and microglia-specific depletion of PQBP1 in primary culture in vitro and mouse brain in vivo shows that PQBP1 is essential for sensing-tau to induce nuclear translocation of nuclear factor κB (NFκB), NFκB-dependent transcription of inflammation genes, brain inflammation in vivo, and eventually mouse cognitive impairment. Collectively, PQBP1 is an intracellular receptor in the cGAS-STING pathway not only for cDNA of human immunodeficiency virus (HIV) but also for the transmissible neurodegenerative disease protein tau. This study characterizes a mechanism of brain inflammation that is common to virus infection and neurodegenerative disorders.

Highlight

PQBP1, a new intracellular receptor for viral infection and neurodegeneration.

PQBP1 functions in brain microglia, which corresponds to dendritic cells or a part of macrophage regulating innate immune system, as an intracellular receptor for HIV cDNA and Tau proteins similarly.



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Department of Biochemical Pathophysiology

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

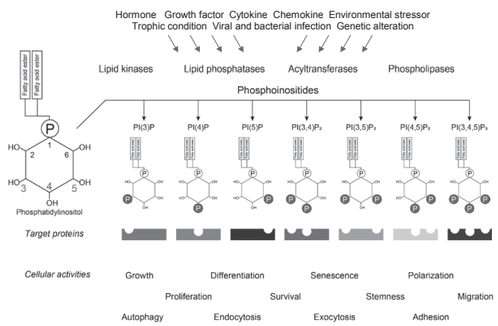

Fig.1 Phosphoinositide

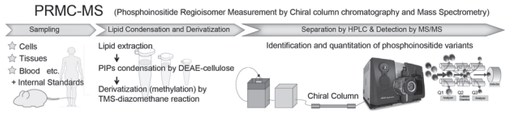

Fig.2 A new method for comprehensive analysis of phosphoinositide

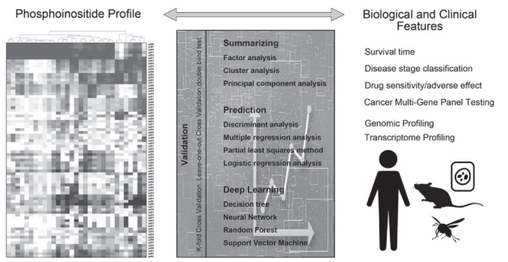

Fig.3 Phosphoinositide variants as molecular signatures for various diseases

Research Projects

1. Comprehensive measurement of phosphoinositide variants

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

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

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

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

2. Phosphoinositide metabolism to maintain femaleness

Mammalian sex is determined by the Y-linked Sry (Sex-determining region Y) gene. The supporting cell precursors of the fetal gonad differentiate into testicular Sertoli cells in the presence of Sry, while the cells differentiate into ovarian granulosa cells in the absence of Sry. These supporting cells commit to sexual differentiation of somatic and germ cells. Recent studies in mice have provided evidence that granulosa vs Sertoli cell fate decision is not necessarily permanent in postnatal life. In the adult ovary, Foxl2 (forkhead box L2) and estrogen receptors are required for maintaining granulosa cell fate by repressing male promoting signals, while in the adult testis, Dmrt1 (doublesex and mab-3 related transcription factor 1) and Sox9 (SRY-box 9) are required for maintaining Sertoli cell fate by repressing female promoting signals. Abolishing female promoting signals leads to loss of granulosa cell fate markers and up-regulation of Sertoli fate markers such as Sox9 and Dmrt1. We found that conditional knockout mouse mutants lacking phosphatidylinositol 3,4,5-trisphosphate (PIP3) phosphatases exhibited female infertility. Histological examination of the mutant ovaries revealed that Sertoli-like cells emerged in the follicles. The female mutant mice had significantly higher levels of testosterone in the serum. Our results demonstrate that PIP3 metabolism plays a key role in cell fate determination towards granulosa cells in the ovaries, and propose a possible etiology of DSD, disorders of sex development.

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Department of Pathological Cell Biology

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

〈**Research Projects**〉

1-1, Analysis of Atg5 -independent alternative autophagy

Atg5 is considered to be essential molecules for the induction of autophagy. However, we found that cells lacking Atg5 can still form autophagosomes/autolysosomes and perform autophagic protein degradation when subjected to certain types of stress. We named this noncanonical autophagy as alternative autophagy or the Golgi membrane-associated degradation (GOMED), because of its morphological and functional similarities with canonical autophagy.

Unlike canonical autophagy, alternative autophagy is originated from Golgi membranes. The involvement of Golgi membranes was demonstrated by the requirement of several Golgi proteins. Immune electron microscopy demonstrated the presence of Golgi proteins on autophagic membranes, confirming the generation of Golgi-derived autophagic structures.

Molecularly, gene silencing analysis demonstrated the important role of ULK1 and BECLIN1. Regarding ULK1, it is first dephosphorylated at Ser^{637}, then phosphorylated at Ser^{746}, and translocated to the Golgi membrane at the early stage of alternative autophagy. At the downstream step, Wipi3 is translocated from the cytosol to the Golgi membrane, and elongated the phagophore membranes.

The substrates degraded by canonical autophagy and alternative autophagy are substantially different. For example, p62 and LC3, which are well-known substrates of canonical autophagy, are not degraded by alternative autophagy, whereas insulin granules are mainly degraded by alternative autophagy and not by canonical autophagy. Furthermore, in reticulocytes, mitochondria are mostly degraded by alternative autophagy, whereas ER and ribosomes are degraded by canonical autophagy. Therefore, the two autophagy pathways degrade different substrates in the same cell, and basically do not compensate each other.

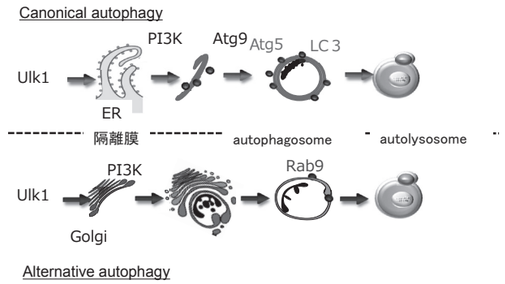

Figure 1. Hypothetical model of autophagy.
There are at least two modes of autophagy, i.e. canonical and alternative autophagy. Canonical autophagy requires Atg5 and is originated from the ER membrane. In contrast, alternative autophagy occurs independently of Atg5 and is originated from the Golgi membrane.

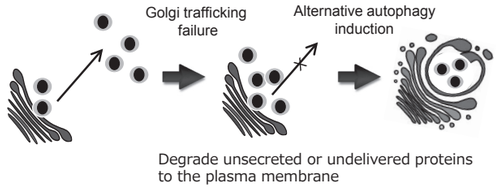

Figure 2. Physological role of alternative autophagy

1-2, Development of small compounds that induce Atg5 -independent alternative autophagy

Because alternative autophagy plays an essential role in maintaining cellular function, the association between alternative autophagy and neurodegenerative diseases is crucial. Therefore, we are trying to develop alternative autophagy-inducing agents using high-throughput screening assay. We now successfully identified one candidate compound, and it showed protective effects against mouse models of neurodegenerative diseases. Therefore, future detailed analyses are expected to clarify the effects of autophagy-inducing therapies on human neurodegenerative diseases.

2, Molecular mechanisms of programmed cell death

Programmed cell death, which is required for the development and homeostasis of metazoans, includes mechanisms such as apoptosis, autophagic cell death, and necrotic death. Apoptosis is carried out by the caspase activation and following substrates digestion. In this year, we focused novel form cell death, namely alternative anoikis. Now, we are searching physiological roles of alternative anoikis.

3, Novel organellar biology

Organelles are small, specialized structures in cells, which play specific roles to regulate various cellular events. The recent rapid development of imaging techniques have clarified the details of organelle dynamics, demonstrating that (1) various functional regions are dynamically formed within organelles, (2) organelle functions are made possible by the comprehensible actions of these functional regions. In this year, we found cross-talk between mitochondria and Golgi apparatus. We also found that various mitochondrila functions are influenced by the Golgi-localized molecules.

Publications

[Original paper]

1. Homeostatic p62 levels and inclusion body formation in CHCHD2 knockout mice. S. Sato, S. Noda, S. Torii, T. Amo, A. Ikeda, M. Funayama, J. Yamaguchi, T. Fukuda, H. Kondo, N. Tada, S. Arakawa, N. Watanabe, Y. Uchiyama, S. Shimizu, N. Hattori. **Human Molecular Genetics** 30: 443-453, 2021
2. Oxidized phospholipids and neutrophil elastase coordinately play critical roles in NET formation. T. Tokuhiro, A. Ishikawa, H. Sato, S. Takita, A. Yoshikawa, R. Anzai, S. Sato, R. Aoyagi, M. Arita, Y. Aratani, S. Shimizu, M. Tanaka, S. Yotsumoto. **Frontiers in Cell and Developmental Biology**, 9:718586, 2021
3. Nickel particles are present in Crohn's disease tissue and exacerbate intestinal inflammation in IBD susceptible mice. H. Matsuda, Y. Nibe-Shirakihara, A. Tamura, E. Aonuma, S. Arakawa, K. Otsubo, Y. Nemoto, T. Nagaishi, K. Tsuchiya, S. Shimizu, A. Ma, M. Watanabe, M. Uo, Ryuichi Okamoto. **Biochem. Biophys. Res. Commun**. 592, 74-80, 2022
4. Molecular mechanisms and biological roles of GOMED. S. Noguchi and S. Shimizu. **FEBS J**. doi: 10.1111/febs.16281, 2021

Department of Developmental and Regenerative Biology

Professor	Hiroshi Nishina, Ph.D.
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	Keiko Kanayama, Ph. D.
	Jing Pu, 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

MKK7-JNK signaling pathway is constitutively activated in mammalian brains and are indispensable for their development and neural functions. However, whether the MKK7-JNK signaling pathway regulates the brain's control of social behavior remains unclear. Here, we show that female mice in which *Mkk7* is deleted specifically in mature neurons (*Mkk7*$^{flox/flox}$*Syn-Cre* mice) give birth to a normal number of pups but fail to raise them due to a defect in pup retrieval. To explore the mechanism underlying this abnormality, we performed comprehensive behavioral tests. *Mkk7*$^{flox/flox}$*Syn-Cre* mice showed normal locomotor functions and cognitive ability but exhibited depression-like behavior. cDNA microarray analysis of mutant brain revealed an altered gene expression pattern. Quantitative RT-PCR analysis demonstrated that mRNA expression levels of genes related to neural signaling pathways and a calcium channel were significantly different from controls. In addition, loss of neural MKK7 had unexpected regulatory effects on gene expression patterns in oligodendrocytes. These findings indicate that MKK7 has an important role in regulating the gene expression patterns responsible for promoting normal social behavior and staving off depression (Figure 1).

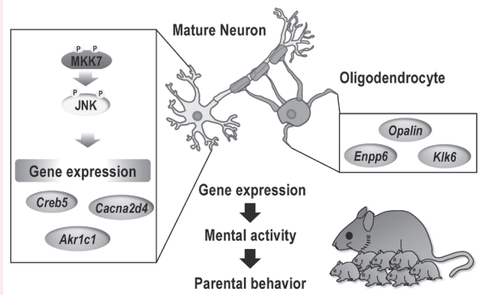

Fig.1.A Schematic model of molecular mechanisms for mouse parental behavior by MKK7-JNK signaling pathway.

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Department of Immunology

Professor — Takeshi Tsubata, M.D., Ph.D.
Assistant Professor — Chizuru Akatsu, Ph.D.
Project Assistant Professor — Cheryl Sophia Sultan, Ph.D.
Lecturer — Ji-Yang Wang, Ph.D.
Researcher — Amin Alborzian Deh Sheikh, Ph.D.

Antibody responses to non-protein antigens such as polysaccharide and nucleic acids play crucial roles in host defense against pathogens, and development of autoimmune diseases. The mechanisms for antibody responses to non-protein antigens are distinct from those to protein antigens (Fig. 1), but are largely unknown. We are elucidating mechanisms for antibody responses to non-protein antigens in normal immunity and self-tolerance to non-protein self-antigens that is involved in prevention of autoimmunity. We are developing new antibody drug for autoimmune diseases and vaccines against cancer and COVID-19.

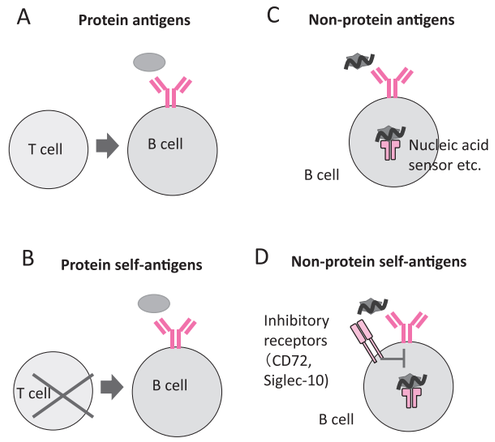

Fig.1 Immune responses and tolerance of B cells to non-protein antigens
When B cells recognize protein antigens, B cells require help from T cells that recognize the same antigens for activation and antibody production (A). Self-reactive T cells are normally absent. As a result, B cells do not respond to protein self-antigens (B). Non-protein antigens such as nucleic acids and polysaccharides activate B cells in the absence of T cell help through T cell-independent mechanisms such as nucleic acid sensors (receptors) (C). Inhibitory receptors such as CD72 and Siglec-10 recognize non-protein self-antigens, and inhibit activation of B cells to non-protein self-antigens thereby maintain self-tolerance even in the absence of regulation by T cells (D).

1. Study on the mechanisms for immune responses and self-tolerance to non-protein antigens

We have demonstrated that inhibitory B cell co-receptors such as CD72 and Siglec-10 are involved in T cell-independent inactivation of B cells reactive to non-protein self-antigens such as the nucleic acid-related antigen Sm/RNP (Akatsu et al. J. Exp. Med. 2016) and sialic acid-containing glycolipids called gangliosides (Alborzian Deh Sheik et al. 2021), respectively, which are involved in development of SLE and GBS, respectively. We further demonstrated that CD72 recognizes ribosome and the complement component C1q (Akatsu et al, in preparation). Ribosome contains RNA, and C1q binds to apoptotic body containing nucleic acids, leading to T cell-independent B cell activation through nucleic acid sensors. CD72 suppresses B cell activation induced by these self-antigens. Because autoantibodies against ribosome and C1q as well as Sm/RNP are SLE-specific autoantibodies, our findings indicate that CD72 suppresses B cell responses to various SLE-specific self-antigens, thereby preventing development of SLE.

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

Glycoproteins and glycolipids on the cell surface form a glycan-rich layer called glycocalyx. In the glycocalyx, glycan-mediated molecular interactions are thought to regulate cellular functions. However, little is known about the details. CD22, an inhibitory co-receptor expressed in B cells, possesses an extracellular lectin domain recognizing sialic acids, and associates with sialylated glycoproteins and glycolipids on the same cell (cis-ligands). We recently showed that interaction of CD22 with cis-ligands in glycocalyx is involved in the quality control of B cells allowing development of signaling-competent B cells (Akatsu et al. Science Signaling 2022, Alborzian Deh Sheikh J. Immunol. 2021, also see Highlight). We also demonstrated in collaboration with Prof. Butcher at the Stanford University that CD22 regulates cell surface expression of the integrin $\alpha 4\beta 7$ involved in lymphocyte homing to gut-associated lymphoid tissue through sialic acid-mediated interaction with $\alpha 4\beta 7$ in glycocalyx (Ballet et al. Nat Immunol. 2021).

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

We are developing new therapy for autoimmune diseases through expansion of regulatory B cells. We also develop vaccines including therapeutic vaccines that induce production of antibodies to the molecules involved in cancer immune checkpoints and vaccines against COVID-19 that maintain effectiveness to variants.

Highlight

Inhibitory B cell co-receptor CD22 paradoxically restores signaling in immune deficient B cells.

CD22 (also known as Siglec-2) is an inhibitory receptor expressed in B cells. CD22 contains an extracellular lectin domain that recognizes sialic acid. CD22 interacts with sialic acid-containing glycoproteins and glycolipids expressed on the same cell (cis-ligands), leading to CD22 homo-clustering and interaction of CD22 with B cell antigen receptor. We demonstrated that CD22 suppresses signaling in B cells during their differentiation to mature B cells, leading to the quality control of B cells through selective maturation of signaling-competent B cells. In immunodeficiencies caused by B cell signaling defects, the CD22-mediated quality control allows selective maturation of B cells with increased antigen receptor expression, thereby restoring B cell signaling function at least in part (Fig. 2) (Akatsu et al. Science Signaling 2022).

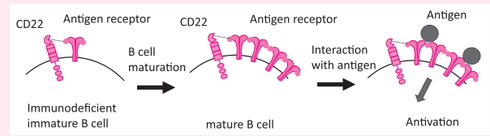

Fig.2 CD22-mediated functional restoration of immunodeficient B cells
When antigens interact with the antigen receptor on B cells, B cells undergo activation and differentiation to antibody-producing cells (plasma cells). Defects in signaling through the antigen receptor cause immunodeficiency. The function of immunodeficient B cells is restored by increasing the amount of antigen receptors during development of mature B cells. This process requires interaction of CD22 with its cis-ligands.

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[original papers]

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Division of Medical Genomics

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

【Main Research Activities】

- We investigated how low birth weight and fetal growth restriction may result from the mother having certain variants of hypertension-related genes.〔*Department of Molecular Epidemiology*〕
- Using the data from genome-wide association study (GWAS) of rheumatoid arthritis, we constructed a polygenic risk score (PRS), which could predict radiographic progression in individuals with rheumatoid arthritis.〔*Department of Genomic Function and Diversity*〕
- We identified specific variations and alterations in genes commonly found in Japanese esophageal squamous cell carcinoma (ESCC) patients.
- We also identified pyruvate dehydrogenase (PDH) component X (PDHX) as a metabolically essential gene for the cell growth of ESCC.〔*Department of Molecular Cytogenetics*〕
- Using sedimentation velocity centrifugation, we fractionated chromatin from cultured cells based on its local compaction states. We showed the local state of chromatin compaction appeared to influence the frequency of RNA polymerase binding, which ultimately regulated the transcription levels of individual genes.〔*Department of Functional Genome Informatics*〕

Department of Molecular Cytogenetics

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Associate Professor	Jun Inoue, Ph.D.
Assistant Professor	Yasuyuki Gen, M.D., Ph.D.
Assistant Professor	Tomoki Muramatsu, Ph.D.

The principal aim of the Department of Molecular Cytogenetics is to understand the molecular mechanisms and to establish practical and useful modalities for the diagnosis and therapy of personalized medicine for cancer and genetic diseases, with the purpose of addressing unmet medical needs.

I. Development of microRNA-based cancer therapeutics

1) Development of anticancer drugs using tumor-suppressive *miR-634*

MicroRNAs (*miRs*) are functional RNAs consisting of approximately 22 nucleotides that repress gene expression by directly binding to the transcripts of multiple target genes. We have previously demonstrated that the introduction of *miR-634*, a tumor-suppressive (TS)-*miR*, into cancer cells efficiently induces cell death by simultaneously targeting multiple genes related to the cytoprotective process. In addition, we have developed nucleic acid anticancer drugs (systemic and topical ointment formulations) using synthetic double-stranded *miR-634* mimic, and demonstrated the therapeutic efficacy and safety of these *miR-634* therapeutics using xenograft mouse model.

In the current study, we found that introduction of *miR-634* into oral squamous cell carcinoma (OSCC) cell line markedly enhanced the efficacy of cisplatin. Furthermore, we newly identified the *cIAP1* gene, which has an essential role in anti-apoptosis, as a *miR-634* target. While the expression of cIAP1 is upregulated by gene amplification in cisplatin-resistant OSCC cells, introduction of *miR-634* overcomes cisplatin resistance by targeting of multiple target genes, including *cIAP1*. We also found that local administration of *miR-634* ointment onto the tumor and systemic administration of cisplatin induced synergistic anti-tumor effects in an OSCC xenograft mouse model (Tran PX et al. Mol Ther - Oncolytics 2022).

2) *miR-766-5p* targeting super-enhancers by downregulating CBP and BRD4

We showed that *miR-766-5p* directly targets CBP and BRD4. Concurrent suppression of *CBP* and *BRD4* cooperatively downregulated *MYC* expression in cancer cells but not in normal cells. Chromatin immunoprecipitation analysis revealed that *miR-766-5p* reduced levels of H3K27ac at *MYC* super-enhancers (SEs) *via* CBP suppression. Moreover, *miR-766-5p* suppressed expression of a BRD4-NUT fusion protein that drives NUT midline carcinoma. *In vivo* administration of *miR-766-5p* suppressed tumor growth in two xenograft models. Targeting SEs using *miR-766-5p*-based therapeutics may serve as an effective strategy for the treatment of MYC-driven cancers (Gen Y et al. Cancer Res.2021).

3) *miR-3140-3p* overcomes acquired resistance to BETis in neuroblastoma (NB)

NB harboring *MYCN* amplification shows a poorer prognosis. As BRD4 drives transcription of *MYCN* in NB cells, BET inhibitors (BETis) are considered useful for NB therapy. However, clinical trials of BETis suggested that early acquired resistance to BETis limits their therapeutic benefit. We revealed that *miR-3140-3p* suppresses tumor cell growth in *MYCN*-amplified NB by downregulating MYCN and MYC through BRD4 suppression. We next disclosed that activated ERK1/2 stabilizes MYCN protein by preventing ubiquitin-mediated proteolysis via phosphorylation of MYCN at Ser62 in BETi-acquired resistant NB cells, thereby attenuating the effects of BETi in these cells. *miR-3140-3p* downregulated MYCN expression by directly targeting the MAP3K3-ERK1/2 pathway in addition to BRD4 suppression, inhibiting tumor cell growth in BETi-acquired resistant NB cells. *miR-3140-3p* has the potential to overcome resistance to BETi in NB. (Liu C et al. Mol Ther - Nucleic Acids. 2021)

II. Identification of *NUF2* missense variant associated with microcephaly and short stature

We report a male patient with a rare *de novo* missense variant in *NUF2*, after trio whole-exome sequencing analysis. The patient presented with microcephaly and short stature, with additional features, such as bilateral vocal cord paralysis, micrognathia and atrial septal defect. *NUF2* encodes a subunit of the NDC80 complex in the outer kinetochore, important for correct microtubule binding and spindle assembly checkpoint. The mutated residue is buried at the calponin homology (CH) domain at the N-terminus of NUF2, which interacts with the N-terminus of NDC80. The variant caused the loss of hydrophobic interactions in the core of the CH domain of NUF2, thereby impairing the stability of NDC80-NUF2. Analysis using a patient-derived lymphoblastoid cell line revealed markedly reduced protein levels of both NUF2 and NDC80, aneuploidy, increased micronuclei formation and spindle abnormality. Our findings suggest that NUF2 may be the first member of the NDC80 complex to be associated with a human disorder (Uehara DT et al. Humu Genet 2021).

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Department of Molecular Genetics

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

BRCA1 and BRCA2 function in DNA double-strand break repair. This function is a guardian against carcinogenesis, while in cancerous tissues, BRCA1 and BRCA2 repair DNA damages caused by anticancer drugs and inhibit the induction of cell death, resulting in resistance to treatment. Based on these findings, synthetic lethal therapy has been developed, and we are conducting research to further advance this therapy. In addition, breast cancer develops in an estrogen-dependent manner, progressing from enhanced survival signaling due to DNA damage repair dysfunction to estrogen-independent proliferative capacity. Therefore, we are investigating the regulation of DNA stability by estrogen and BRCAs to elucidate the mechanism of mammary carcinogenesis and to develop novel therapies for breast cancer using these mechanisms.

1. BRCA2 represses the transcriptional activity of pS2 by E2-ER α

BRCA2 gene mutations or deficiencies increase the risk of carcinogenesis in tissues where estrogen acts, such as the mammary gland and ovary. In addition to estrogen uptake, BRCA2 expression increases in the S phase of the cell cycle and largely contributes to DNA damage repair associated with DNA replication. However, the role of BRCA2 in estrogen induction remains unclear. An expression plasmid was created to induce BRCA2 activation upon the addition of estradiol by introducing mutations to the binding sequences for the transcription factors USF1, E2F1, and NF-κB within the promoter region of BRCA2. Then, the estrogen receptor alpha (ER α) sites of the proteins that interact with BRCA2 upon the addition of estradiol were identified. Both proteins were bound by the helical domain of BRCA2 and activation function-2 of the ER α, suggesting that this binding may regulate the transcriptional activity of pS2, a target gene of the estradiol-ER α, by suppressing the binding of SRC-1, a coactivator required for activation of the transcription factor.

2. The BRCA2 missense mutation K2497R suppressed self-degradation and increased ATP production and cell proliferation

Breast cancer susceptibility gene 2 (BRCA2) mediates genome maintenance during the S phase of the cell cycle, with important roles in replication stress, centrosome replication, and cytokinesis. In this study, we showed that a small heat shock protein, HSP27, interacted with and participated in the degradation of BRCA2 in estrogen-treated MCF-7 cells. BRCA2 degradation reportedly requires ubiquitination of the C-terminal region; thus, fragments of amino acid (aa) residues 2241–2940 were produced and assayed for their degradation following cycloheximide (CHX) treatment. The results showed that aa 2491–2580 affected the degradation of BRCA2, especially lysine (Lys) 2497. Furthermore, the K2497A/R mutation increased ATP production and the proliferation of DLD-1 (BRCA2 knockout) cells compared to the cells expressing wild-type BRCA2-FLAG. Notably, a single residue, Lys2497, affected BRCA2 degradation, and K2497R is reportedly a missense mutation in hereditary breast cancer. The results showed that the accumulation of BRCA2, which avoided degradation, enhanced cell proliferative capacity.

3. Development of novel synthetic lethal therapy for chemo-resistant tumors

Genome plasticity during cancer progression and therapy allows cancer cells to acquire chemoresistance. These mechanisms should be analyzed as clinically serious problems to overcome cancer therapeutic resistance. Hereditary breast and ovarian cancer (HBOC) by genetic defects in BRCA genes has dysfunctional DNA homologous recombination (HR) repair. The tumors show sensitive response to DNA damaging agents as synthetic lethality, so that a PARP inhibitor-based treatment has been provided in Japan. However, various types of acquired chemoresistance mechanisms were reported, resulting in treatment difficulty. Clinical strategies for most of the acquired chemo-resistances have not been established yet.

Therefore, we screened chemical compounds to explore novel factors to sensitize tumor cells combined with DNA damaging agents such as PARP inhibitor and etoposide. We utilized known compounds for drug-repositioning, and performed our original high-throughput screening to assess DNA damage repair machinery. So far, we found various candidates for synthetic lethal therapy and proceeded further investigation. As a typical example, we found steroid compounds sensitized tumor cells combined with Topoisomerase II inhibitor, etoposide. Steroids have not formed DSBs in cells alone, while those increased DSBs combined with etoposide, resulted in cellular sensitization. Several data have indicated that the transcriptional activity of steroids potentiate etoposide-induced DNA damage. We will figure out the more detailed mechanisms and develop those for clinical applications such as drug repositioning.

Highlight

- BRCA2 interacted with ER α via E2 induction.
- The α-helical domain (HD) of BRCA2 bound to the ER α.
- BRCA2 bound to the ER α activation function-2 (AF-2) domain and inhibited pS2 transcriptional activity via E2-ER α.

The results of this study showed that BRCA2 can downregulate the expression of pS2 by repressing the binding of SRC-1 to ER α. In doing so, BRCA2 did not affect the binding of E2 to ER α or the binding of E2-ER α to oligonucleotides. It was suggested that BRCA2 regulate runaway cell division in steps as DNA damage repair and repression of transcriptional activity by E2-ER α. (Figure)

Fig.1 BRCA2 inhibited the binding of ERa to SRC-1, thereby suppressing the transcriptional activity of pS2.

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Department of Molecular Epidemiology

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

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

1. COL17A1 germline variant p.Ser1029Ala and mucosal malignant melanoma: An autopsy study

Collagen type XVII α1 (COL17A1) encodes a hemidesmosomal protein at the epidermal dermal junction and its variants are implicated in blistering skin diseases. Recent experiments in rodents revealed that Col17a1 has critical roles in stem cells of epidermal origin and in melanoma carcinogenesis. In the present study, it was investigated whether germline variants in COL17A1 are associated with skin cancer and other cancer types using indexed consecutive autopsy cases from the Japanese Geriatric Single Nucleotide Polymorphism database (n=2,343; mean age, 80 years). The database included 12 patients with skin cancer. A total of 53 COL17A1 missense variants on an exome chip were analyzed. One variant, p.Ser1029Ala (rs118166857), which had a minor allele frequency of 1.0%, exhibited a nominal positive sign of association with skin cancer [Fisher's exact P=0.002, odds ratio (OR)=16.93, 95% CI: 4.44 64.64]. This variant was detected in 2/2 patients with mucosal malignant melanoma (mMM) and 1/3 patients with extramammary Paget's disease, and in none of the patients with non melanoma cancer, e.g., squamous cell and basal cell carcinoma. Other cancer types were searched in the database and the p.Ser1029Ala variant was indicated to be nominally associated with breast cancer (P=0.006, OR=4.17, 95% CI: 1.72 10.11). In the two mMM cases, targeted exome sequencing of 55 cancer predisposing genes (including tumor protein 53, BRCA1/2 and mismatch repair genes) detected no apparent pathogenic variants, but revealed variants of unknown significance in axin 2, DNA directed polymerase ζ catalytic subunit and contactin 6. Since COL17A1 provides a niche for melanocyte stem cells, it was hypothesized that the p.Ser1029Ala variant in the COL17A1 ectodomain may affect the microenvironment, e.g., the cell competition. This is a working hypothesis generated from human autopsy cases and warrants further epidemiological and molecular biological validation.

2. Placenta mediates the effect of maternal hypertension polygenic score on offspring birth weight: a study of birth cohort with fetal growth velocity data

Background

Low birth weight (LBW) and fetal growth restriction are associated with the development of cardio-metabolic diseases later in life. A recent Mendelian randomization study concluded that the susceptibility of LBW infants to develop hypertension during adulthood is due to the inheritance of hypertension genes from the mother and not to an unfavorable intrauterine environment. Therein, a negative linear association has been assumed between genetically estimated maternal blood pressure (BP) and birth weight, while the observed relationship between maternal BP and birth weight is substantially different from that assumption. As many hypertension genes are likely involved in vasculature development and function, we hypothesized that BP-increasing genetic variants could affect birth weight by reducing the growth of the placenta, a highly vascular organ, without overtly elevating the maternal BP.

Methods

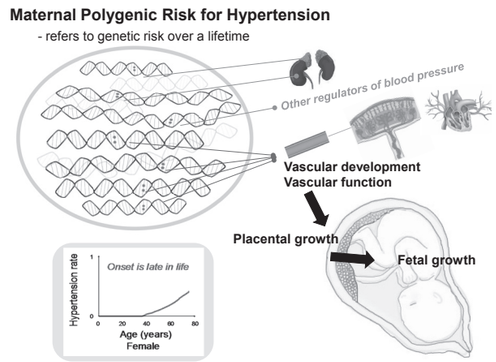
Using a birth cohort in the Japanese population possessing time-series fetal growth velocity data as a target and a GWAS summary statistics of BioBank Japan as a base data, we performed polygenic score (PGS) analyses for systolic BP (SBP), diastolic BP, mean arterial pressure, and pulse pressure. A causal mediation analysis was performed to assess the meditation effect of placental weight on birth weight reduced by maternal BP-increasing PGS. Maternal genetic risk score constituted of only "vasculature-related" BP single nucleotide polymorphisms (SNPs) was constructed to examine the involvement of vascular genes in the mediation effect of placental weight. We identified gestational week in which maternal SBP-increasing PGS significantly decreased fetal growth velocity.

Results

We observed that maternal SBP-increasing PGS was negatively associated with offspring birth weight. A causal mediation analysis revealed that a large proportion of the total maternal PGS effect on birth weight was mediated by placental weight. The placental mediation effect was remarkable when genetic risk score was constituted of "vasculature-related" BP SNPs. The inverse association between maternal SBP PGS and fetal growth velocity only became apparent in late gestation.

Conclusions

Our study suggests that maternal hypertension genes are strongly associated with placental growth and that fetal growth inhibition is induced through the intrauterine environment established by the placenta.



A higher maternal genetic risk of hypertension results in reduced growth of placenta, a vascular organ, which in turn causes reduced fetal growth.

Publications

[Original Paper]

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Department of Functional Genome informatics

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Associate Professor Yohei Sasagawa, Ph.D.
Assistant Professor Mariko Yamane, Ph.D.
Technical assistant Ikuko Maeda

Research Objects

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

Research activities

(1) The origin of hair follicle stem cells

A research group led by Dr. Rizuko Morita and Dr. Hirofumi Fujiwara of the Laboratory for Tissue Microenvironment (Fujiwara lab) at the RIKEN Center for Biosystems Dynamics Research, and Dr. Tetsutaro Hayashi and Dr. Mika Yoshimura of the Laboratory for Bioinformatics Research (RIKEN BiT) at the RIKEN Center for Frontier Biosciences, has identified the origin of hair follicle stem cells. The research group led by Dr. Tetsutaro Hayashi and Dr. Yoshi Yoshimura of the RIKEN BiT team has clarified the source of hair follicle stem cells. They also revealed that hair follicles are generated by a "telescopic model," a morphogenetic model that differs from known mechanisms.

We aimed to elucidate the developmental origin of hair follicle stem cells by combining long-term ex vivo live imaging. The development of mouse hair follicles is observed overtime at the single-cell level and single-cell transcriptome analysis. We found that developing hair follicles are compartmentalized into telescope-like cylinders that elongate during development. We also found that stem cells originate from one of these compartments. Our laboratory collaborated with RIKEN BiT to conduct single-cell RNA-seq experiments and analyze the data.

(2) Epigenome changes and brain dysfunction

Our laboratory collaborated with researchers Dr. Ayumi Yamada and Dr. Yoichi Makai at the Cellular Memory Laboratory, Dr. Tetsutaro Hayashi and Dr. Mika Yoshimura at the RIKEN BiT, and Associate Professor Takae Hirasawa, Teikyo University.

We used mouse models to study brain dysfunction in Kliegstra syndrome (KS), an inherited neuropsychiatric disorder that causes developmental delays, impaired intelligence, and autism-like symptoms. KS is an inherited neuropsychiatric disorder that causes developmental delays, intelligence deficits, and autism-like symptoms and is thought to be caused by abnormalities in the epigenome that prevent the normal transcription of disease-related genes, resulting in brain dysfunction. Therefore, the research team analyzed mice with a heterozygous deletion of Ehmt1 as a model mouse for KS. Emhm1, which coded the histone methyltransferase GLP, is the gene responsible for KS. As a result, we showed that behavioral abnormalities, such as decreased activity and increased anxiety, can be ameliorated by supplementation of GLP, which is reduced after birth. In addition, the brain of Ehmt1 heterozygous mice showed an inflammatory state caused by the activation of microglia. Using single-cell RNA-seq, we found that microglial inflammation is one of the causes of the phenotype in the brain of KS model mice. Our laboratory collaborated with RIKEN BiT for the single-cell RNA-seq experiments and data analysis.

(3) Physically measuring the relationship between gene coarseness and expression.

In collaboration with Dr. Satoru Ishihara at Fujita Medical School and Dr. Hayato Yamashita at Osaka University, we have elucidated the mechanism regulating gene activity strength using human cells.

The normal functioning of genes is essential for human health, and their abnormalities cause various diseases. Traditionally, genes were thought to be regulated by an on/off "switch," with two patterns: working or not working. However, the reality is that gene expression is regulated in an infinite number of steps, from zero to the maximum. To elucidate the nature of the "dial" that enables this variable regulation, we focused on nucleosomes, the cylindrical structures around which genes are wrapped, and developed a new method of nucleosome analysis using an ultracentrifuge. The analysis method revealed that the function of genes is suppressed when several nucleosomes are clustered in a "dense" manner, while genes work strongly when they are scattered in a "sparse" manner. In other words, we can conclude that the degree of nucleosome densification is the "dial" that determines the strength of gene function. Therefore, the findings revealed in this study are expected to lead to new strategies for understanding human health and disease. Our laboratory collaborated with RIKEN BiT for the sequencing experiments and data analysis.

(4) Analysis of oocyte senescence

Dr. Tomoya Kitajima, Dr. Tatsuhei Mishina at Lab. for Chromosome Segregation, RIKEN BDR and collages conducted whole gene expression (transcriptome) analysis of female mouse oocytes at the single-cell level during the early, middle, and late reproductive lifespan to capture gene expression changes associated with oocyte aging. The results revealed that dietary restriction (calorie restriction) might inhibit oocyte aging.

The research group used single-cell RNA sequencing to profile changes in gene expression during aging in oocytes, the source of mouse oocytes. This analysis revealed that large-scale gene expression changes occur in oocytes at the late stage of reproductive life. In addition, dietary restriction, which inhibits individual aging, altered the expression of genes associated with aging and reduced the number of proteins involved in oocyte aging. These results indicate that maternal age and dietary restriction affect aging-related changes in oocytes.

The results of this study provide essential information for understanding the mechanism of oocyte aging and are expected to contribute to the development of technologies for predicting abnormalities in the number of chromosomes in oocytes. Our laboratory collaborated with RIKEN BiT for the sequencing experiments and data analysis.

Research achievements

Original Paper

1. Ayumi Yamada, Takae Hirasawa, Kayako Nishimura, Chikako Shimura, Naomi Kogo, Kei Fukuda, Madoka Kato, Masaki Yokomori, Tetsutaro Hayashi, Mana Umeda, Mika Yoshimura, Yoichiro Iwakura, Itoshi Nikaido, Shigeyoshi Itohara, Yoichi Shinkai. Derepression of inflammation-related genes link to microglia activation and neural maturation defect in a mouse model of Kleefstra syndrome. iScience. 2021. 24(7) 102741-102741
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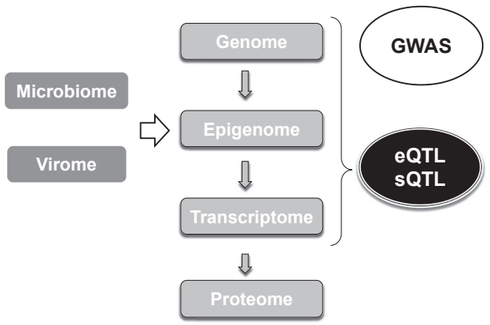
Department of Genomic Function and Diversity

Professor **Yuta Kochi**
Associate professor **Satomi Mitsuhashi**
Assistant professor **Mahoko Ueda**

Research objectives

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



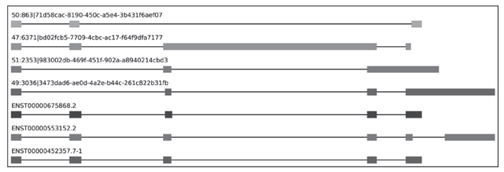
Research activities

1. Integration of GWAS and eQTL/sQTL studies

Majority of GWAS loci identified in complex traits are now considered to be eQTL or sQTL where genetic variants regulate expression levels of genes or alternate splicing. Therefore, to interpret the results of GWAS for dissecting the mechanism of disease, it is essential to integrate the results of GWAS and 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.

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

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



2. Functional analysis of GWAS genes

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

Some RNAs transcribed from repetitive sequences derived from retrotransposon function in innate immunity, and they are involved in the pathophysiology of not only immune diseases but also other multifactorial diseases. In fact, we identified Ervpb1 in human endogenous retrovirus (HERV) sequences in an Alzheimer's disease locus by utilizing long-read sequencing technologies (*Int J Mol Sci*. 2021). We will further seek retroelement-derived RNAs involved in the etiology of complex traits.

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 : Jun Inamo (PhD researcher), Miwako Okawa (Secretary)

Publications

Original articles

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6. Mitsuhashi S, Nakagawa S, Sasaki-Honda M, Sakurai H, Frith MC, Mitsuhashi H. Nanopore direct RNA sequencing detects DUX4-activated repeats and isoforms in human muscle cells. ***Hum Mol Genet***. 2021;30(7):552-63.
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Review articles

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Department of Medical Science Mathematics

Professor Tatsuhiko Tsunoda
Junior Associate Professor Fuyuki Miya

Research Summary

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

Research Projects

1. Methodology for original analysis with deep learning

We research the ability of convolutional neural networks to process not only images, but also non-image data, particularly omics data. They could be applied to analysis of image data such as pathological and biomolecular images, analysis of omics data, and integrated analysis of both datasets. As an example, we developed a unique technique for transforming omics data to look like an image and apply convolutional neural networks to it, so that we can distinguish cancer types from omic data. We also developed a technique to analyze the inner workings of neural network for identify how it discriminates them (Figure) **[1]**. By doing so, we discovered a new signaling system that indicates the individuality of cancer types. This means that we were able to show that deep learning can be used to make new scientific discoveries.

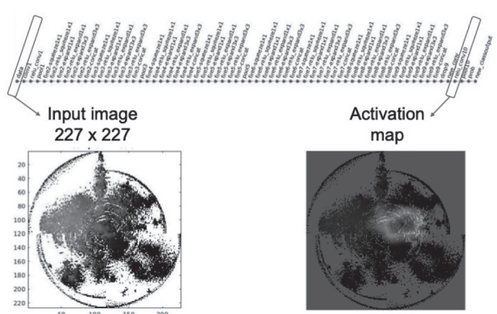

Figure. Analyze what deep learning sees when identifying cancer types [1].

2. Understand Tumor-microenvironments

By investigating the relationship between cancer cell populations and their microenvironment, such as the immune system, we can model and predict response to treatment, side effects, and the acquisition of resistance on an individual basis. This time, we discovered a new cluster in colorectal cancer **[2]**. Further detailed analysis revealed that the population characteristics of cancer cells and their relationship with the microenvironment, such as immunity, vary greatly among clusters. Similarly, in gastric and renal cancers, we have elucidated the impact of the tumor-microenvironment on therapeutic response and prognosis **[3-6]**.

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

One type of next-generation sequencing data analyses is WES analysis. This approach has made a particularly strong impact in the studies of Mendelian diseases using family data. We have identified many disease-causing genes of intractable diseases, e.g. neurodegenerative diseases, by using our original experimental methods and analysis pipeline that were developed to achieve both high coverage and accuracy **[7-10]**.

4. Results of other research projects [11-18]

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Joint Research Division

Systems Biology for Intractable Diseases

Laboratory for Integrated Research Projects on Intractable Diseases

Advanced Technology Laboratories

Joint Research Division, Department of Precision Health

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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 of diseases. It is 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 before their onset, resulting in extending healthy life. Recently, "pre-disease" is focused on a novel therapeutic target to maintain health. 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 by detecting slight abnormalities in the body more quickly and easily.

1. Analysis of immune response

We have generated 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. 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. 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 events occurring in the process of pathological progress.

Highlight

The most abundant immunoglobulin (antibody) in our body is immunoglobulin A (IgA), and it has been known that IgA is secreted into the intestinal lumen and is important for the elimination of pathogens. However, although selective IgA deficiency is the most common in human immunodeficiency, no serious symptoms were observed. Thus, the precise function of IgA remains unclear. We have generated IgA-deficient mice, and found that in these mice, the intestinal flora of the small intestine was distorted, and an excessive immune cell activation especially in the ileum, causing ileal inflammation. Thus, IgA contributes to the homeostasis of the intestinal flora of the ileum to prevent inflammation.

(Gut 2022, https://www.tmd.ac.jp/english/press-release/20210527-1/, https://www.youtube.com/watch?v=Z8OYt-9BUSg)

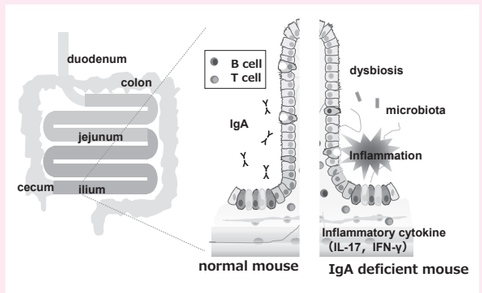

Figure 1. IgA deficiency induces ileitis
The small intestine is composed of duodenum, jejunum and ileum (left). IgA deficiency causes distortion of the gut microbiota, especially in the ileum, and inflammatory cytokines are secreted from T cells, resulting in in inflammation of the ileum (right).

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 mechanisms of the intestinal sensing network and the crosstalk such as the gut-brain mediated by orally administrated-food and medicine. We also analyze other crosstalk such as the gut-skin axis.

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

4. Clinical application for monitoring of predisposition of diseases

We aim to establish a method for detecting slight changes (predisposition of diseases) before the onset of diseases and try to develop foods and medicine to prevent and treat these disorders. Based on these basic researches, we are trying 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.

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Systems Biology for Intractable Diseases

Visiting Professor　Seiya Imoto

It is getting clearer that pathogenesis of intractable disease is a state that deviates from an integrated system's control to abnormal situation where multiple genes are affecting one another intricately. The recent advances in biomedical research have been producing large-scale, ultra-high dimensional, ultra-heterogeneous omics data through the advanced technologies such as genome sequencing and proteome analysis. Also, not only our own genome, but also dysbiosis of commensal microbiota is known to be related with various diseases including IBD, Parkinson disease and other intractable diseases. The aim of this section is to clarify the biological mechanisms and their failures in the system by applying computational strategy for systems biology and by analyzing these omics data using supercomputer. It is expected that key molecules of the diseases will be searched by the systems biology analysis of molecular pathways and networks related to the diseases which could not be analyzed in the traditional approaches. This section is collaborating with various laboratories in Medical Research Institute for understanding the pathogenesis of the diseases toward drug discovery and new therapy development.

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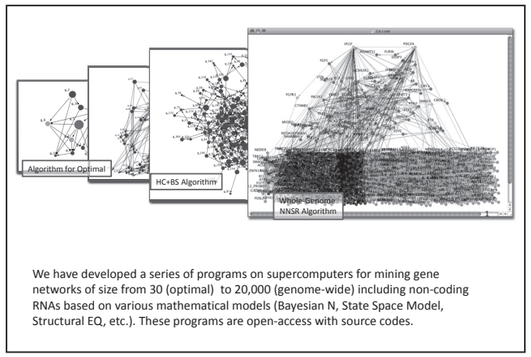
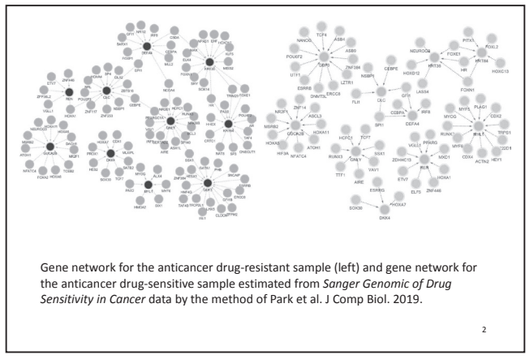


We have developed a series of programs on supercomputers for mining gene networks of size from 30 (optimal) to 20,000 (genome-wide) including non-coding RNAs based on various mathematical models (Bayesian N, State Space Model, Structural EQ, etc.). These programs are open-access with source codes.



Gene network for the anticancer drug-resistant sample (left) and gene network for the anticancer drug-sensitive sample estimated from *Sanger Genomic of Drug Sensitivity in Cancer* data by the method of Park et al. J Comp Biol. 2019.

2

Laboratory for Integrated Research Projects on Intractable Diseases

IBD project3 Laboratory for Integrated Research Projects on Intractable Diseases

Professor Shigeomi SHIMIZU
Toshiaki OHTEKI.

Summary

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

Research Outcome

Result 1. In order to elucidate the relationship between alternative autophagy and IBD, we generated various alternative autophagy knockout mice. Some mice were susceptible to DSS-induced colitis, suggesting protective role of alternative autophagy on colitis. We are analyzing its mechanisms using organoids generated from these knockout mice.

Result 2. We are trying to develop molecularly targeted anti-IBD agents based on the induction of alternative autophagy. To meet this objective, we have established a high-throughput assay system that can monitor alternative autophagy–inducing activity. Using this assay, we screened a low–molecular weight compound library and successfully identified 20 candidate compounds.

Result 3. We identified the deposition of nickel particles within Crohn's disease tissue specimens by Synchrotron radiation-induced X-ray fluorescence spectroscopy and X-ray absorption fine structure analysis. Nickel particles exacerbated dextran sulfate sodium-induced colitis in mice, illustrating that nickel particle ingestion may worsen Crohn's disease.

Publications

1. Nickel particles are present in Crohn's disease tissue and exacerbate intestinal inflammation in IBD susceptible mice. H. Matsuda, Y. Nibe-Shirakihara, A. Tamura, E. Aonuma, S. Arakawa, K. Otsubo, Y. Nemoto, T. Nagaishi, K. Tsuchiya, S. Shimizu, A. Ma, M. Watanabe, M. Uo, Ryuichi Okamoto. **Biochem. Biophys. Res. Commun**. 592, 74-80, 2022
2. Molecular mechanisms and biological roles of GOMED. S. Noguchi and S. Shimizu. **FEBS J**. doi: 10.1111/febs.16281, 2021

Core research project for the implementation of precision medicine of head and neck and esophageal squamous cell carcinoma

Project leader Johji Inazawa
Collaborators Takahiro Asakage, Tohru Ikeda, Masanobu Kitagawa, Tatsuhiko Tsunoda, Toshihiro Tanaka, Hiroyuki Harada, Satoshi Miyake, Naoto Fujiwara, Sadakatsu Ikeda, Keiichi Morita, Akira Takemoto, Kousuke Tanimoto, Tomoki Muramatsu

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

Research outcome

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

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

2) Clinical sequencing analysis

Clinical sequencing analyses were performed in 495 tumor samples including 21 ESCCs and 48 HNSCCs by December 31, 2021.

3) Identification of *PDHX* as a metabolic target for ESCC

We identified that PDHX (pyruvate dehydrogenase [PDH] component X) is involved in energy production through the activity of the PDH complex in mitochondria, and its expression is essential for ESCC cell proliferation. *PDHX* gene is located adjacent to *CD44* gene, a cancer stem cell marker, within chromosome 11p13 region and is upregulated by co-amplification, indicating that the PDHX contributes to cancer stemness. Furthermore, we found that the administration of the PDH inhibitor CPI-613 markedly suppressed tumor growth in the ESCC xenograft mouse model (Inoue J, Kishikawa M et al. Cancer Sci. 2021).

4) *miR-766-5p* Targets Super-Enhancers by downregulating CBP and BRD4.

We previously identified *miR-766-5p* as a miRNA that downregulated *MYC* expression and inhibited cancer cell growth *in vitro*. Recently, we showed that *miR-766-5p* directly targets CBP and BRD4. Concurrent suppression of CBP and BRD4 cooperatively downregulated MYC expression in cancer cells but not in normal cells. Chromatin immunoprecipitation analysis revealed that *miR-766-5p* reduced levels of H3K27ac at *MYC* super-enhancers (SEs) via CBP suppression. Moreover, *miR-766-5p* suppressed expression of a BRD4-NUT fusion protein that drives NUT midline carcinoma. *In vivo* administration of *miR-766-5p* suppressed tumor growth in two xenograft models. Targeting SEs using *miR-766-5p*-based therapeutics may serve as an effective strategy for the treatment of MYC-driven cancers (Gen Y et al. Cancer Res. 2021).

5) Integrative genome-wide analyses reveal the transcriptional aberrations in Japanese ESCC

Japanese ESCC cases were clustered into two mutational signatures: an APOBEC-associated signature and an age-related signature. In imprinted genes, DNA methylation was aberrant in gene promoter regions and correlated well with gene expression profiles. Nonsynonymous single-nucleotide variants and allelic expression imbalance were detected frequently in *FAT* family genes. Our integrative genome-wide analyses, including DNA methylation and allele-specific gene expression profiles, revealed

altered gene regulation of imprinted genes and *FAT* family genes in ESCC (Takemoto A et al. Cancer Sci. 2021).

6) *miR*-therapeutics for plastic thyroid cancer (ATC), an advanced head and neck cancer

We demonstrated the therapeutic efficacy of topical administrated tumor suppressive *miR-634* ointment in xenograft mouse models of head and neck cancers, including anaplastic thyroid cancer (ATC) and oral squamous cell carcinoma (OSCC). Furthermore, we found that topical administration of *miR-634* ointment significantly enhanced the antitumor effects of tyrosine kinase inhibitors lenvatinib in ATC or cisplatin in OSCC by targeting multiple gene related with cytoprotective processes in tumor cells. Thus, *miR-634* ointment is expected to become a therapeutic modality to maximize the therapeutic effect of chemotherapy in advanced head and neck cancer (Kishikawa M et al. Biochem Biophys Rep. 2021, Tran PX et al. Mol Ther - Oncolytics. 2022).

Publications

1. Tran PX, Inoue J, Harada H, Inazawa. Potential for reversing miR-634- mediated cytoprotective processes to improve efficacy of chemotherapy against oral squamous cell carcinoma. Mol Ther Oncolytics. 2022 Mar 8: 24: 897-908
2. Gen Y, Muramatsu T, Inoue J, Inazawa J: miR-766-5p targets super-enhancers by downregulating CBP and BRD4. Cancer Res. 2021Oct 15. 81:5190-5201. doi: 10.1158/0008-5472.CAN-21-0649. Epub 2021 Aug 5.
3. Takemoto A, Tanimoto K, Mori S, Inoue J, Fujiwara N, Noda T, Inazawa J: Integrative genome-wide analyses reveal the transcriptional aberrations in Japanese esophageal squamous cell carcinoma. Cancer Sci. 2021 Oct. 112:4377-4392. doi: 10.1111/cas.15063. Epub 2021 Aug 12.
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5. Kishikawa M, Inoue J, Hamamoto H, Kobayashi K, Asakage T, Inazawa J: Augmentation of lenvatinib efficacy by topical treatment of miR-634 ointment in anaplastic thyroid cancer. Biochem Biophys Rep. 2021 May 9. 26:101009. doi: 10.1016/j.bbrep.2021.101009. eCollection 2021 Jul.
6. Inoue J*, Kishikawa M*, Tsuda H, Nakajima Y, Asakage T, Inazawa J: Identification of PDHX as a metabolic target for esophageal squamous cell carcinoma. Cancer Sci. 2021 Jul;112:2792-2802. doi: 10.1111/cas.14938. Epub 2021 May 24. *equally contributed

DOHaD research toward preventive and preemptive approach against chronic non-communicable diseases

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

Summary

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

Research Outcome

1. There is a concern that a large percentage of pregnant Japanese women are underweight and do not have adequate nutritional status. We have assessed the dietary quality of pregnant women using the Nutrient-Rich Food Index (NRF9.3) along with Dietary Inflammation Index (DII). The results showed that vitamins and dietary fiber were the nutrients that contributed most to individual differences in dietary quality, and vegetables and fruits were the most important food groups. However, diet quality was not simply associated with weight-related traits.
2. The birth weight of offspring born to mothers with a high polygenic score (PGS) for systolic blood pressure (SBP) is low. It has been inferred to be that maternal hypertension reduces fetal growth. However, there is no negative correlation between actual maternal blood pressure and birth weight. We showed that the effect of maternal SBP-PGS on birthweight was mediated by decreased placental growth, not maternal hypertension, using PGS analysis, bioinformatics, and causal mediation analysis. Furthermore, we found that the association between maternal SBP-PGS and deceleration of fetal growth velocity only appeared later in gestation. Our finding is highly important for the perinatal management of fetal growth arrest in the late gestation. Given that many fetal growth arrest or restriction (FGR) are late-onset and difficult to diagnose, maternal SBP-PGS will be useful to the screening for late-onset FGR high-risk groups, regardless of the presence or absence of maternal hypertension.
3. During pregnancy, the placenta-derived extracellular vesicles (EVs) increases in circulating blood, and the levels of EV-encapsulated nucleic acids, especially miRNAs (EV-miRNAs), are considered useful as markers reflecting fetal and placental development. We focused on two EV-miRNAs (miR127-3p and miR-26b-5p), whose circulating levels are relatively high, and investigated the relationship between the levels of these EV-miRNAs in mid- and late- pregnancy and fetal and placental growth in normal vaginal deliveries at term. We found that the negative association between EV-miR-127-3p and BWGA z-score and the positive association between miR-26b-5p and birth weight were observed at mid-pregnancy miRNA levels, but disappeared or weakened in late-pregnancy, suggesting that the association with fetal and placental growth is trimester-specific. It suggests that the association with fetal and placental development is period-specific.

Publications

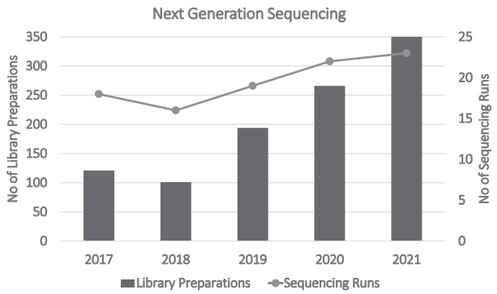
[Original Paper]
1. Imai C, Takimoto H, Fudono A, Tarui I, Aoyama T, Yago S, Okamitsu M, Sasaki S, Mizutani S, Miyasaka N, Sato N. Application of the Nutrient-Rich Food Index 9.3 and the Dietary Inflammatory Index for Assessing Maternal Dietary Quality in Japan: A Single-Center Birth Cohort Study. Nutrients. 13(8):2854. 2021.
2. Sato N, Fudono A, Imai C, Takimoto H, Tarui I, Aoyama T, Yago S, Okamitsu M, Mizutani S, Miyasaka N. Placenta mediates the effect of maternal hypertension pol;ygenic score on offspring birth weight: a study of birth cohort with fetal growth velocity data. BMC Medicine. 19(1):260, 2021
3. Fudono A, Imai C, Takimoto H, Tarui I, Aoyama T, Yago S, Okamitsu M, Muramatsu M, Sato N, Miyasaka N. Trimester-specific associations between extracellular vesicle microRNAs and fetal growth. J Matern Fetal Neonatal Med. 1-7. doi: 10.1080/14767058.2021.2000598. Online ahead of print. 2021

Press Release/News
1. 2 Minute Medicine. 2021.11.15. “Maternal genetic risk of hypertension associated with reduced placental weight” : https://www.2minutemedicine.com/maternal-genetic-risk-of-hypertension-associated-with-reduced-placental-weight/
2. EurekAlert! The Global Souce for Science News. 2021.12.03. “The placenta-the smoking gun in cardiovascular disease”: https://www.eurekalert.org/news-releases/936785
3. The Medical News. 2021.12.06. “New therapeutic targets for the treatment and prevention of hypertension, cardiovascular diseases”: https://www.news-medical.net/news/20211206/New-therapeutic-targets-for-the-treatment-and-prevention-of-hypertension-cardiovascular-diseases.aspx

Advanced Technology Laboratories

Genome Laboratory

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



Followings are the achievements in 2021.

1. Sequencing analyses

A total of 12,506 samples from 1,441 researchers were sequenced in the year of 2021. Among them 7,699 (62%) samples were requested by researchers outside the medical Research Institute (see below). Analysis by using next generation sequencing equipment (Ion PGM and Ion S5) has been started in 2013 and 23 runs were done in the year of 2021. Library preparation service for next generation sequencing has been started in 2015 and 382 samples were done in the year of 2021.

2. Equipment under the management of the Genome Laboratory.

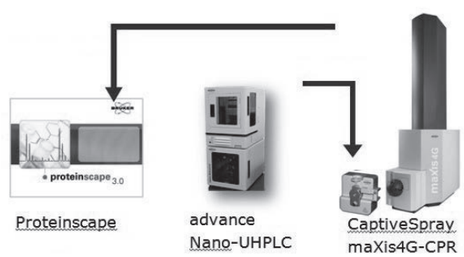
DNA sequencer (ABI3130xl) × 2, Next generation sequencer (Ion PGM, Ion S5), PCR machine (ABI7900) × 5, Flow Cytometer, Luminescent Plate Reader, Ice Machine, Centrifuge, Ion One Touch system, DNA/RNA shearing system, and others.

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

1. Short DNA/RNA heteroduplex oligonucleotide interacting proteins are key regulators of target gene silencing

Ken Asada, Fumika Sakaue, Tetsuya Nagata, Ji-chun Zhang, Kie Yoshida-Tanaka, Aya Abe, Makiko Nawa, Kazutaka Nishina, and Takanori Yokota

Nucleic Acids Res. 2021 May 21; 49(9): 4864–4876.

2. CSE1L promotes nuclear accumulation of transcriptional coactivator TAZ and enhances invasiveness of human cancer cells

Shunta Nagashima,[1] Junichi Maruyama,[1,*] Kaori Honda,[2] Yasumitsu Kondoh,[2] Hiroyuki Osada,[2] Makiko Nawa,[3] Ken-ichi Nakahama,[4] Mari Ishigami-Yuasa,[5] Hiroyuki Kagechika,[5,6] Haruhiko Sugimura,[7] Hiroaki Iwasa,[1] Kyoko Arimoto-Matsuzaki,[1] Hiroshi Nishina,[8] and Yutaka Hata[1,9,*]

J Biol Chem. 2021 Jul; 297(1): 100803.

Laboratory of Genome Editing for Biomedical Research

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

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

Laboratory of Anatomy and Cell Function

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

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

<<Common equipment>>

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

<<seminars and demonstrations>>

A user of Confocal laser microscope and Laser Capture Microdissection is required to attend a seminar for learn of the correct way to use.

In this fiscal year, seminars were held on the following dates.

Confocal laser microscope…5/12, 11/30 (Carl Zeiss)
Laser Capture Microdissection…4/28 (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 the current year. Now the Laboratory is equipped with basic and state-of-the-art research instruments, including high-speed cell sorters (MoFlo XDP), confocal laser scanning microscopes (FV10i-W for time-lapse images, and FV10i-DOC for one shot images).

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

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

The number of overall use cases was 257 in the year of 2021.

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. In this year, we received orders from two universities. 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. We also undertake tests for mycoplasma contamination. We continued the preservation service for the biological samples using a large liquid nitrogen tank (Fig.2), which was requested by many laboratories on campus.
In this fiscal year, the steering committee chairman was replaced but the work is properly operated.

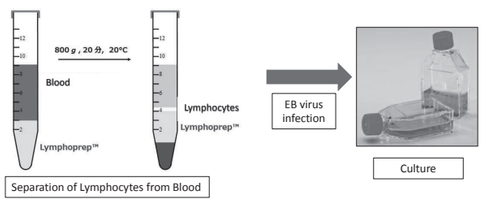


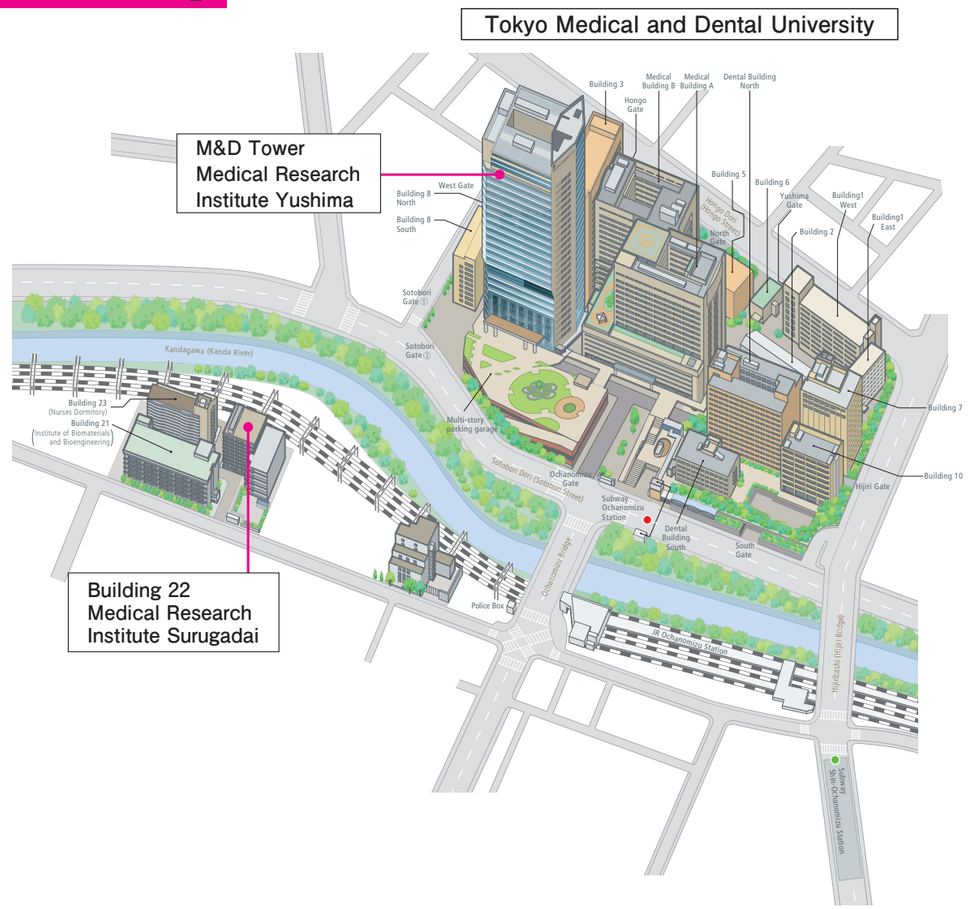
Fig. 1 Establishing Human B Lymphoblasticcell Lines using EBV

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

Laboratory for Structure Analysis

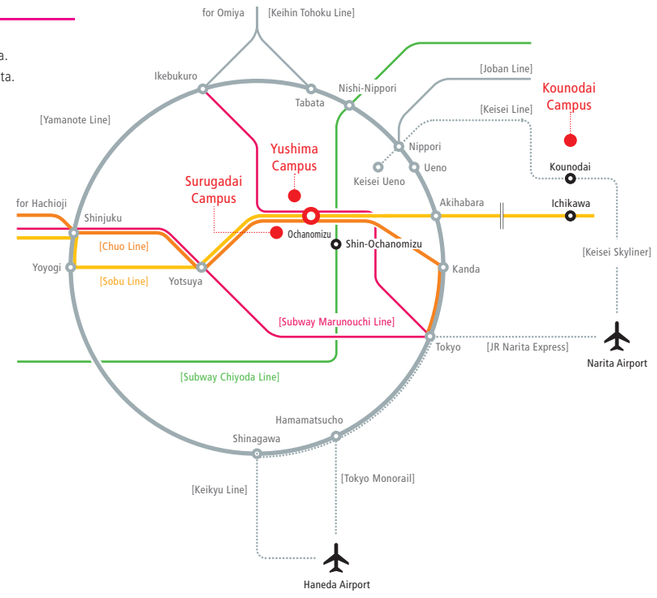
The Laboratory for Structure Analysis is equipped with a high-brilliancy X-ray generator (Rigaku MicroMax007HF) and an imaging plate X-ray detector (Rigaku R-AXIS VII) for the structure determination of biological macromolecules. The laboratory is also equipped with a dynamic light scattering (DLS) instrument (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.

Access Map



Nearest Stations

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



ANNUAL REPORT 2022

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