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ANNUAL REPORT 2017

Tokyo Medical and Dental University

Medical Research Institute / Tokyo Medical and Dental University

Annual Report 2017

2017

Annual Report

Medical Research Institute

Tokyo Medical and Dental University

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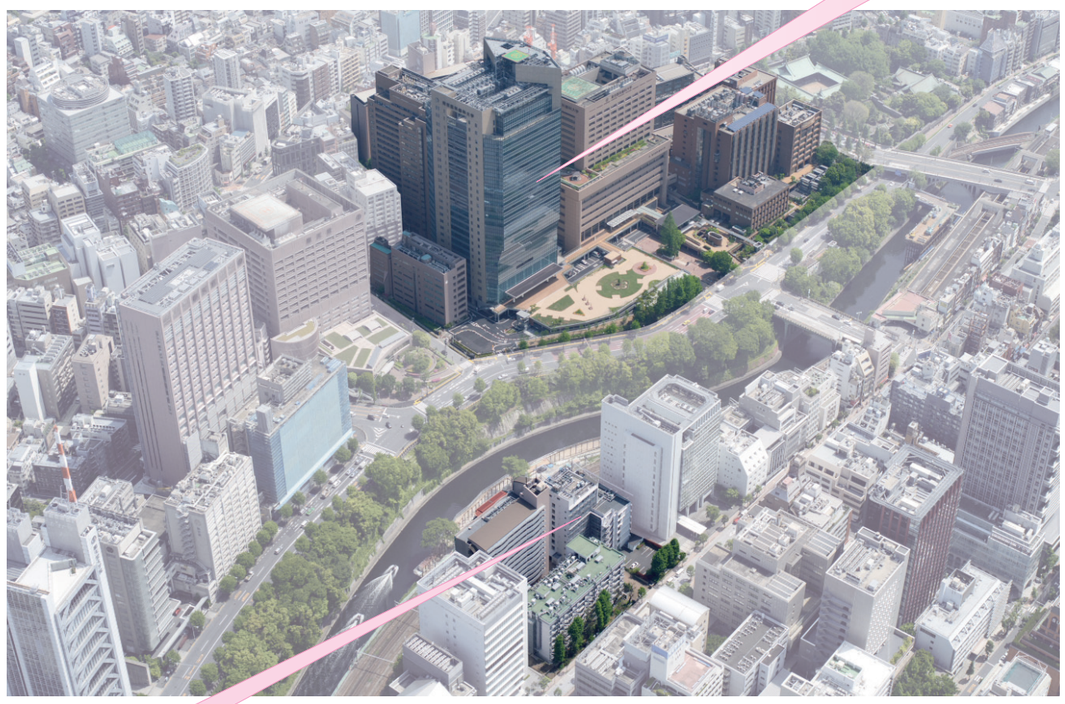
Medical Research Institute

Yushima Area

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

Medical Research Institute
Department of 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 Molecular Pathogenesis, Department of Epigenetics, Department of Stem Cell Regulation, Department of Neuropathology, Department of Structual Biology, Department of Biodefense Research, Department of Stem Cell Biology, Department of Genomic Pathology, Department of Molecular Epidemiology, Frontier Research Unit Laboratory of Gene Expression, Frontier Research Unit Redox Response Cell Biology, Frontier Research Unit Laboratory of Oxygen Biology, Tenure Track Research Unit Department of Cellular and Molecular Medicine, Project Research Unit, Administrative Office



Surugadai Area

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

Medical Research Institute
Project Research Unit

Medical Research Institute

Director
- Fumitoshi Ishino
- Faculty Meeting

Research Divisions

Division of Advanced Molecular Medicine	Department of Molecular Medicine and Metabolism		
	Department of Molecular Pharmacology	(under consideration)	
	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		
	Tenure Track Research Unit Department of Cellular and Molecular Medicine		
	Frontier Laboratory: Skeletal Molecular Pharmacology		
Division of Pathophysiology	Department of Neuropathology	Professor	Hitoshi Okazawa
	Department of Pathological Biochemistry	(under consideration)	
	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	Professoror	Akinori Kimura
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 Biochemical Genetics	(under consideration)	
	Department of Genomic Pathology	Professor	Shumpei Ishikawa
	Department of Epigenetics	Professor	Fumitoshi Ishino
	Department of Medical Science Mathematics	Professor	Tatsuhiko Tsunoda
	Frontier Research Unit Laboratory of Gene Expression		
	Project Reserch Unit		
Division of Integrative Research	System Biology for Intractable Diseases	Visiting Professors	Satoru Miyano and Seiya Imoto
	Laboratory for Integrated Research Projects on Intractable Diseases		
Advanced Technology Laboratory	Genome Laboratory		
	Laboratory of Cytometry and Proteome Research		
	Laboratory of Recombinant Animals		
	Laboratory of Anatomy and Cell Function		
	Bioresource Laboratory		
	Laboratory of Structural Biology		
	Stem Cell Laboratory		
Administrative Office	General Affairs Section		

Highlight

Drs. Tsubata and Akatsu at the Department of Immunoloby and Drs. Ito and Numoto at the Department of Structural Biology elucidated a novel mechanism that inhibits development of systemic lupus erythematosus (SLE), a prototype of systemic autoimmune diseases. This study was supported by Japan Society for the Promotion of Science Grants-in-Aid for Scientific Research, and was published in the Journal of Experimental Medicine.

The number of the patients with SLE is relatively large among autoimmune diseases, and there are around 60000-100000 patients in Japan. SLE is characterized by production of autoantibodies to nuclear antigens, which play a crucial role in development of this disease. Patients with SLE are treated with corticosteroids and immune suppressants. However, these treatments cause adverse effects such as metabolic diseases and infection. Thus, development of new therapies based on the pathogenesis of SLE is awaited as such therapies may be more effective without generating adverse effects.

Immune cells including B lymphocytes (B cells) express various nucleic acid (NA) sensors that recognize NAs and activate immune cells. NA sensors recognize microbial NAs and are involved in induction of immune responses to microbes. However, some of these NA sensors can recognize self-NAs as well as microbial NAs, and are involved in development of SLE through production of antibodies to nuclear antigens. Among various NA sensors, TLR7 plays a crucial role in development of SLE by recognizing Sm/RNP, one of self-NAs, and inducing anti-Sm/RNP antibody production.

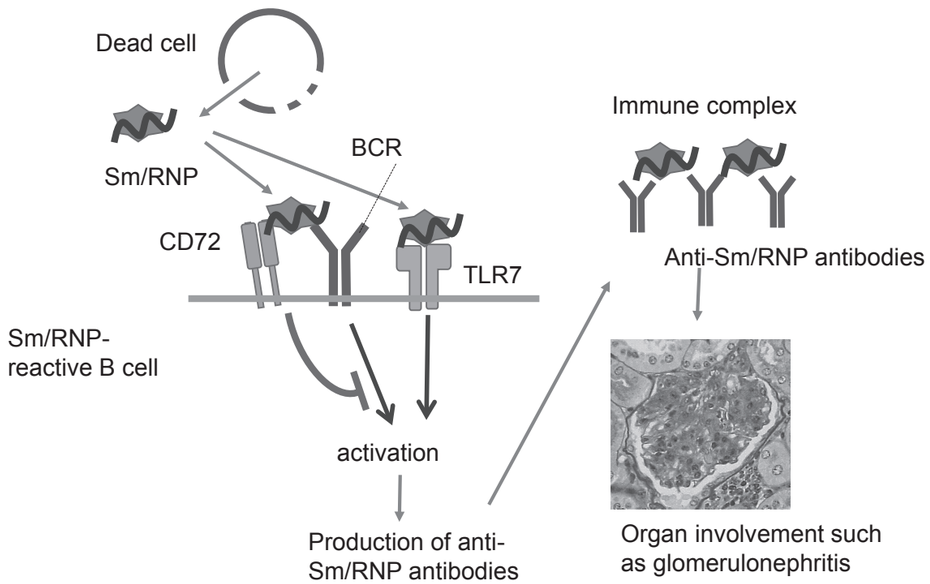


Figure. CD72 inhibits immune response to the nuclear self-antigen Sm/RNP that plays a crucial role in development of SLE.
When Sm/RNP, a complex of nucleic acids and nuclear proteins, is released from dead cells, Sm/RNP is recognized by both TLR7 and B cell antigen receptor (BCR) in B cells that produce anti-Sm/RNP antibodies, leading to activation of Sm/RNP-reactive B cells and production of anti-Sm/RNP antibodies crucial for development of SLE. The inhibitory receptor CD72 binds to Sm/RNP, and inhibits activation of Sm/RNP-reactive B cells thereby preventing development of SLE.

This study elucidated that the inhibitory receptor CD72 binds to Sm/RNP, and inhibits TLR7-dependent activation of B cells that recognize Sm/RNP as an antigen, thereby inhibiting production of anti-Sm/RNP antibody and preventing development of SLE.

Localization of TLR7 in endosome plays a role on discrimination of microbial NAs from self-NAs. Self-NAs derived from dead cells are mostly present in a form of free NAs and are rapidly degraded by nucleases in body fluid. In contrast, microbial NAs are resistant to nucleases because they are located inside of the microbes, and are therefore exposed to NA sensors after microbes are endocytosed by host cells. Complexes of self-NAs and nuclear proteins such as Sm/RNP are resistant to nucleases, and are recognized by endosomal NA sensors upon endocytosis, leading to immune responses including autoantibody production. Our results demonstrated involvement of the inhibitory receptor CD72 in discrimination of self-NAs complexed with proteins from microbial NAs. Furthermore, CD72 suppresses immune response to self-NAs involved in development of SLE but not other immune responses including those to microbes. Thus, CD72-mediated immune suppression may be a good target to develop more specific treatment of SLE without adverse effects.

Inter-University Research Network for Trans-Omics Medicine

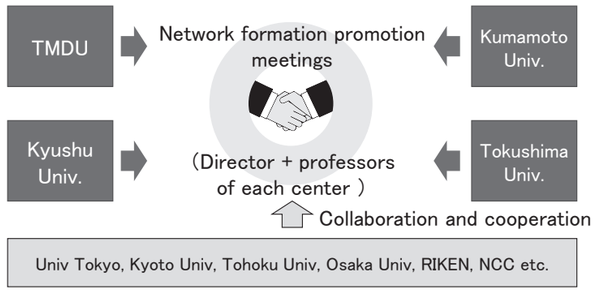
Since April, 2016, Medical Research Institute at Tokyo Medical and Dental University has been promoting the "Inter-University Research Network for Trans-Omics Medicine Project" aiming to establish a trans-omics research education hub. We are carrying out this project in cooperation with the Joint Usage/Research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

Aim of the Project

* By stimulating genome/epigenome technology, we establish a new research platform for trans-omics.
* We also train experts to deal with several big data for trans-omics.

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

Network formation promotion meetings

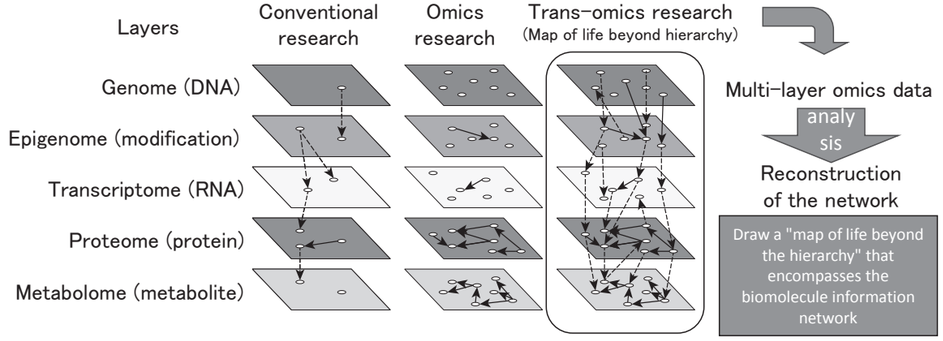
* The first meeting (kick-off meeting)
  Date: June 25, 2016
  Place: Medical Institute of Bioregulation, Kyushu University
* The second meeting
  Date: November 2, 2016
  Place: Science Café at Biomedical Research Station, Kyushu University
* The second meeting (expanded)
  Date: November 2, 2016
  Place: Seminar Room 105 Biomedical Research Station, Kyushu University

Joint research symposium

* The first symposium "Trans-Omics: New Approaches in Biology and Medicine"
  Date: November 2-3, 2016
  Kyushu University Station-I for Collaborative Research, Auditorium

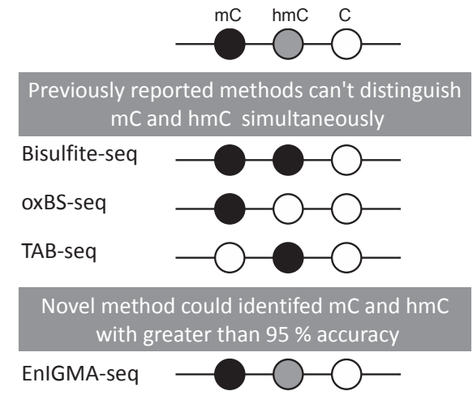
In order to truly understand biological phenomena and disease mechanisms, it is necessary to reconstruct the information network we woven from multiple hierarchical omics data to understand cell strategies (trans-omics research). However, the protocol of trans-omics research does not exist, nor the platform. 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, intractable disease research institute acquires omics data mainly on three layers of genomics, epigenomics and transcriptomics. We promote creative research that can be a model of trans-omics research by systematically conducting research through cooperation with other three centers. Especially in epigenomics research, we are establishing new hydroxymethylcytosine analysis method and plan to standardize this method and integrate it into the protocol of trans-omics research.



Development of new epigenome analysis method

Acquisition of accurate information on the epigenome, which is a key to regulating gene expression, is an important layer in multi-omics research. Among them, analysis of methylation modification of cytosine in genomic DNA is one of its major parts. Recently it has been revealed that methylcytosine (mC) is oxidized to hydroxymethylcytosine (hmC) by Tet enzyme, which is an important intermediate in the active demethylation process, and hmC itself is also important for transcriptional regulation. However, previouse technologies for identification of hmC at a single base resolution, such as oxBS and Tab-seq can't simultaneously identify mC and hmC on the same molecule. Though it is possible to estimate the ratio with mC by comparing with the result of conventional bisulfite method, it is not possible to directly analyze these three modification states. For this reason, these methods have many problems including insufficient quantitativeness. Thus, in this research project, we developed an experimental method based on a new principle that identifies mC, hmC, and unmodified cytosine (C) simultaneously at single base resolution. We devised a method to distinguish between mC and hmC by utilizing the specificity that DNMT1, a maintenance methylation enzyme, methylates cytosine only in the opposite strand of hemi-mC. We named this method Enzyme-Assisted Identification Genome Modification Assay (EnIGMA). Indeed, mC, hmC and C could be identified simultaneously with greater than 95 % accuracy with this method.



Publications

Kawasaki Y. *et al.* A novel method for the simultaneous identification of methylcytosine and hydroxymethylcytosine at a single base resolution. Nucleic Acids Res (2017) **45** (4): e24.

Division of Advanced Molecular Medicine

Aim and Scope

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

【Molecular Cell Biology】

- WDR26 plays a negative role in β-catenin degradation in the Wnt signaling pathway.
- WNK signaling pathway is involved in neural development via Lhx8 gene expression.

【Molecular Neuroscience】

- Gene cassette knock-in in mammalian and zygotes by enhanced MMEJ.
- Posterior Purkinje cells in an SCA5 mouse models are vulnerable to the synergistic effect of loss of β-III spectrin and GLAST.

【Biodefense Research】

- Discovery of human common monocyte progenitor (cMoP).
- Identification of colitogenic macrophages and their epigenetic induction mechanisms.

【Bio-informational Pharmacology】

- Using cardiomyocytes derived from human diseased iPS cells, we clarified the pathogenesis of Brugada syndrome.
- Genetic risk score based on the genome analysis of Japanese atrial fibrillation patients were established.
- Nos1ap, whose gene was related to sudden cardiac death, was found to induce arrhythmogenesis via oxidative stress.

【Stem Cell Regulation】

- Increases of hippocampal dendritic spine density was observed in histone demethylase-gene hypomorphic mice displaying neurodevelopmental disorders-like behavior.
- Sox17-downstream adhesion molecules were found to contribute to the maintenance of hematopoietic cell clusters containing HSCs in the midgestation AGM region.
- C6 glioma stem cells were found to escape from 5- aminolevulinic acid-based photodynamic detection, which could be overcome by iron-chelation.

【Structural Biology】

- The 1.2Å crystal structure of CD72 was determined.
- Some compounds were found to affect the speed of aggregation of Huntingtin.

Department of Molecular Cell Biology

Professor Hiroshi Shibuya
Associate Professor Toshiyasu Goto
Assistant Professor Atsushi Sato

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

Roles of WDR26 in the canonical Wnt signaling.

Wnt signaling plays important roles in multiple developmental events during embryogenesis. Canonical Wnt signaling is initiated by binding of the Wnt ligand to the cell-surface Frizzled and transmembrane LRP complex. This leads to the membrane recruitment and activation of Dishevelled (DVL), which inactivates the APC/Axin/GSK-3 complex in the cytoplasm, responsible for the degradation of β-catenin. As a result, β-catenin accumulates in the cytoplasm, translocates to the nucleus and associates with Tcf transcription factors, which activate the Wnt target genes. In *Xenopus*, Wnt signaling accompanied by β-catenin nuclear localization at the dorsal side is an important for axis formation during early embryogenesis. Ventral over-expression of *Xwnt-8* or *β-catenin* induces a secondary axis and promotes expression of Wnt target genes, such as *Siamois*, *Xnr3* and *Xtwn*. On the other hand, inhibition of canonical Wnt signaling induces head formation on neural stages. Axin1 contains four conserved regions known as a regulation of G-protein signaling (RGS) domain, which binds to APC, at the N-terminus; a dishevelled and axin (DIX) domain at the C-terminus; and binding domains of GSK-3β and β-catenin in the center region. In the canonical Wnt pathway, Axin1 is a component of the β-catenin destruction complex that negatively controls Wnt signaling. Axin1 down-regulates the amount of cytoplasmic β-catenin, to function as a tumor suppressor gene, and several mutations of Axin1 have been identified in tumor cell lines. In *Xenopus* development, Axin1 functions as a ventralizing gene. Axin1 is a multifunctional gene and its function is dependent on binding partners.

To identify novel proteins that may bind to Axin, we performed a high-throughput analysis of proteins that co-immunoprecipitated with human Axin1 in HEK 293 cells using direct nanoflow liquid chromatography-coupled tandem MS (LC-MS/MS). We identified WDR26 as a candidate protein that may physically interact with Axin1. WDR26 contains several protein-interacting domains: the LisH (lis homology domain); the CTLH (C-terminal to LisH motif) domain; and a WD40 repeat domain. A previous study suggested that WDR26 contributes to the MAPK signaling pathway. However, there are no reports that WDR26 is associated with the Wnt signaling pathway. In the yeast *Saccharomyces cerevisiae*, nine glucose-induced degradation-deficient (GID) genes (GID1–GID9) were isolated . The GID complex, which comprises GID1–GID9 except for GID6, acts as a polyubiquitination enzyme in yeast. Eight vertebrate homologs that share high similarities of domain architecture to yeast GID complex genes have been identified. The following are the yeast GID complex genes and their corresponding vertebrate homologs: GID1/RanBP9; GID2/Rmnd5; GID3/UBE2H; GID4/C17ors39; GID5/ARMc8; GID7/WDR26; GID8/TWA1; and GID9/MAEA. Recent studies showed that RMND5 and ARMc8 promote ubiquitination in vertebrates, but it is still unknown whether other vertebrate homologs including WDR26 are associated with the ubiquitination pathway.

We investigated roles of WDR26 and Axin1 in the canonical Wnt signaling pathway, and we performed several analyses to elucidate the mechanism of β-catenin degradation with WDR26, and obtained the following new results.

1. The interaction of ectopically expressed hWDR26 with hAxin1 was confirmed in HEK 293T cells. We found that the N-terminal region including LisH domain was responsible for binding to Axin1. Conversely, WDR26 bound to Axin1 at the central region including GSK3β-binding domain.
2. In *Xenopus*, expression of *xWDR26* was gradually localized to the neural region at the early neurula stage. In the late neurula and tadpole stages, *xWDR26* was strongly expressed in the anterior neural region (Figure 1A).
3. The injection of *xWDR26*-MO (morpholino oligo) into dorso-animal blastomeres of eight-cell embryos, knockdown of xWDR26, reduced both head formation at the tadpole stage (Figure 1B).
4. When *Xwnt-8* mRNA is injected into the ventral sides of four-cell embryos, the target genes of Wnt signaling are induced. Ventral injection of *xWDR26*-MO increased the expression of Wnt target genes that were induced by co-injection of *Xwnt-8* mRNA. Co-injection of *xWDR26* mRNA containing the MO-targeted site with 5-mismatched sequences was restituted the increasing of Wnt target genes by *xWDR26*-MO.
5. The expression of WDR26 reduced the amount of β-catenin protein in cultured cells in a dose-dependent manner, and the knockdown of *WDR26* by siRNA increased the amount of endogenous β-catenin protein in cultured cells, especially in Wnt-stimulated cells.
6. We found that WDR26 did not bind to β-catenin, although Axin1 binds to WDR26. This suggests that WDR26 controls the stability of β-catenin through binding with Axin1. The expression of Wnt target genes induced by ventral injection with *Xwnt-8* mRNA was decreased by co-injection with *xWDR26* mRNA, but not by mRNA of the LisH domain-deleted construct, and the expression of the LisH domain-deleted construct hardly reduce the amount of β-catenin protein. These suggest that binding between WDR26 and Axin is necessary for β-catenin degradation.
7. Although the ubiquitination of x β-catenin was only slightly increased by co-transfection of *xAxin1* alone, the co-transfection of both *xWDR26* and *xAxin1* highly increased the ubiquitination of x β-catenin. These results suggest that WDR26 regulates the ubiquitination of β-catenin for its degradation, and that binding of WDR26 and Axin is important for this ubiquitination.

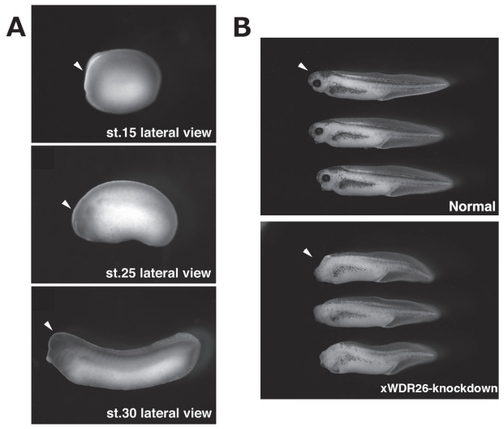


Fig.1

These results suggest that WDR26 plays a negative role in β-catenin degradation with Axin1/WDR26 binding in the Wnt signaling pathway.

Publications

1. Fukuzono, T., Pastuhov, S. Iv., Fukushima, O., Li, C., Hattori, A., Iemura, S., Natsume, T., Shibuya, H., Hanafusa, H., Matsumoto, K. and Hisamoto, N. (2016). Chaperone complex BAG2–HSC70 regulates localization of Caenorhabditis elegans leucine-rich repeat kinase LRK-1 to the Golgi. **Genes Cells** 21, 311-324.
2. Kii, I., Sumida, Y., Goto, T., Sonamoto, R., Okuno, Y., Yoshida, S., Kato-Sumida, T., Koike, Y., Abe, M., Nonaka, Y., Ikura, T., Ito, N., Shibuya, H., Hosoya, T. and Hagiwara, M. (2016). Selective inhibition of the kinase DYRK1A by targeting its folding process. **Nat Commun.** 22, 11391.
3. Goto, T., Matsuzawa, J., Iemura, S., Natsume, T. and Shibuya, H. (2016). WDR26 is a new partner of Axin1 in the canonical Wnt signaling pathway. **FEBS Lett.** 590, 1291-1303.

Department of Molecular Neuroscience

Professor Kohichi Tanaka
Associate Professor Tomomi Aida
Assistant Professor Saeko Ishida
Assistant Professor Yuichi Hiraoka (2016/5/1~)

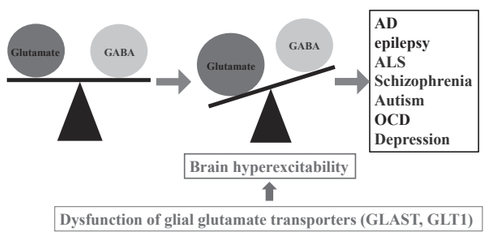
The final goal of our research is to understand molecular, cellular, and neuronal ensemble mechanisms underlying higher order brain functions including learning and memory. For that purpose, we combine molecular genetics, physiological and behavioral methods. The laboratory also studies the mechanism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

Clinical phenotypes of spinocerebellar ataxia type-5 (SCA5) and spectrin-associated autosomal recessive cerebellar ataxia type-1 (SPARCA1) are mirrored in mice lacking β-III spectrin (β-III-/-). One function of β-III spectrin is the stabilization of the Purkinje cell-specific glutamate transporter EAAT4 at the plasma membrane. In β-III-/- mice EAAT4 levels are reduced from an early age. In contrast levels of the glutamate transporter GLAST, expressed in Bergmann glia, only fall progressively from 3 months onwards. Here we elucidated the roles of these two glutamate transporters in cerebellar pathogenesis mediated through loss of β-III spectrin function by studying EAAT4 and GLAST knockout mice as well as crosses of both with β-III-/- mice. Our data demonstrate that EAAT4 loss, but not abnormal AMPA receptor composition, in young β-III-/- mice underlies early Purkinje cell hyper-excitability and that subsequent loss of GLAST, superimposed on the earlier deficiency of EAAT4, is responsible for Purkinje cell loss and progression of motor deficits. Yet the loss of GLAST appears to be independent of EAAT4 loss, highlighting that other aspects of Purkinje cell dysfunction underpin the pathogenic loss of GLAST. Finally, our results demonstrate that Purkinje cells in the posterior cerebellum of β-III-/- mice are most susceptible to the combined loss of EAAT4 and GLAST, with degeneration of proximal dendrites, the site of climbing fibre innervation, most pronounced. This highlights the necessity for efficient glutamate clearance from these regions and identifies dysregulation of glutamatergic neurotransmission particularly within the posterior cerebellum as a key mechanism in SCA5 and SPARCA1 pathogenesis.

Glutamate transporter dysfunction leads to neuropsychiatric diseases



We investigated the cytoprotective effect of geranylgeranylacetone (GGA) on RGCs degeneration using a normal tension glaucoma (NTG) mouse model, which lacks GLAST. Three-week-old GLAST+/− mice were given oral administration of GGA at 100, 300, or 600 mg/kg/day or vehicle alone, and littermate control mice were given vehicle alone for 14 days, respectively. The number of RGCs of GLAST+/− mice significantly decreased, as compared to that of control mice. RGC loss was significantly suppressed by administration of GGA at 600 mg/kg/day, compared with vehicle alone. Following GGA administration, HSP70 was significantly upregulated together with reduction in the activities of caspase-9 and -3. Our studies highlight HSP70 induction in the retina is available to suppress RGC degeneration, and thus GGA may be applicable for NTG as a promising therapy.

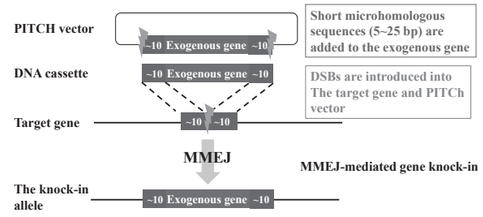
2. Development of genome editing technologies

Although CRISPR/Cas enables one-step gene cassette knock-in, assembling targeting vectors containing long homology arms is a laborious process for high-throughput knock-in. We recently developed the CRISPR/Cas-based precise integration into the target chromosome (PITCh) system for a gene cassette knock-in without long homology arms mediated by microhomology-mediated end-joining.

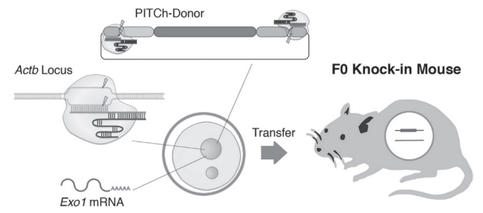
Here, we identified *exonuclease 1* (*Exo1*) as an enhancer for PITCh in human cells. By combining the *Exo1* and PITCh-directed donor vectors, we achieved convenient one-step knock-in of gene cassettes and floxed allele both in human cells and mouse zygotes. Our results provide a technical platform for high-throughput knock-in.

PITCh system (The HR-independent knock-in strategy)

PITCh: precise integration into targeted chromosome



Generation of gene cassette knock-in mice by the enhanced PITCh system



Publications

[Original papers]

1. Aida, T., Nakade, S., Sakuma, T., Ishikubo, H., Usami, T., Aizawa, H., Yamamoto, T., Tanaka, K. Gene cassette knock-in in mammalian cells and zygotes by enhanced MMEJ. *BMC Genomics* 17:979, 2016
2. Dong, Z., Shinmei, Y., Dong, Y., Inafuku, S., Fukuhara, J., Ando, R., Kitaichi, N., Kanda, A., Tanaka, K., Noda, K., Harada, T., Chin, S., Ishida, S. Effect of geranylgeranylacetone on the protection of retinal ganglion cells in a mouse model of normal tension glaucoma. *Heliyon* 2. e00191, 2016.
3. Perkins, EM., Suminaite, D., Clarkson, YL., Lee, SK., Lydon, AR., Rothstein, JD., Wyllie, DJ., Tanaka, K, Jackson, M. Posterior cerebellar Purkinje cells in an SCA5/SPARCA1 mouse models are especially vulnerable to the synergistic effect of loss of β-III spectrin and GLAST. *Hum Mol Genet* 25. 4448-44461, 2016.
4. Marsan, E., Ishida, S., Schramm, A., Weckhuysen, S., Muraca, G., Lecas, S., Liang, N., Treins, C., Pende, M., Roussel, D., Le Van Quyen, M., Mashimo, T., Kaneko, T., Yamamoto, T., Sakuma, T., Mahon, S., Miles, R., Leguern, E., Charpier, S., Baulac, S. Depdc5 knockout rat: A novel model of mTORopathy. Neurobiol Dis 89. 180-189, 2016.

Department of Biodefense Research

Professor	Toshiaki Ohteki, Ph.D.
Junior Associate Professor (to Dec. 14)	Nobuyuki Onai, Ph.D.
Adjunct Lecturer (from Dec. 15)	Nobuyuki Onai, Ph.D.
Adjunct Lecturer (JST PREST)	Taku Sato, Ph.D.
Assistant Professor	Yusuke Nakanishi, Ph.D.
Project Junior Assistant Professor	Jumpei Asano, Ph.D., Mihoko Kajita, Ph.D.
Research Fellow (SONY)	Tomohiko Nakamura
Research Technician	Shoko Kuroda, Kisho Shiseki, Rumiko Nakamura
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 **mononuclear phagocytes** (dendritic cells and macrophages), **tissue stem cells**, and their functional interplay in the immunological and non-immunological organs, such as skin and intestine. 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 mononuclear phagocytes

1) Discovery of a novel source of mononuclear phagocytes

In 1968, Drs. Ralph van Fruth and Zanvil A. Cohn proposed a concept of mononuclear phagocytes that include monocytes and macrophages. In 1973, Dr. Ralph Steinman discovered dendritic cells (DCs), thereby redefining the mononuclear phagocytes as a population consisting of monocytes, macrophages and also DCs. It has been recently continuing epoch-making discoveries in the field of mononuclear phagocytes and their functions are now beyond classical Immunology and rather extend to broad life phenomenon, e.g. tissue development/regeneration, wound-healing, and establishment of various inflammatory diseases (**Fig. 1**).

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

Based on these achievements in mouse, we have been trying to identify human progenitors of mononuclear phagocytes, and most recently succeeded to identify human cMoP (in revision). Human cMoP gives rise to only monocytes but not other hematopoietic cells including DCs (**Fig. 2**). Given that monocytes and monocyte-derived macrophages cause a variety of inflammatory disorders, including metabolic syndromes and tumor development (**Fig. 1**), our studies shed light on possible therapeutic applications for infectious diseases, cancers and autoimmune diseases.

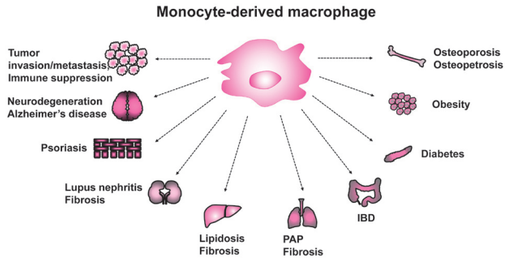


Fig.1 Role of monocyte-derived macrophage in disease

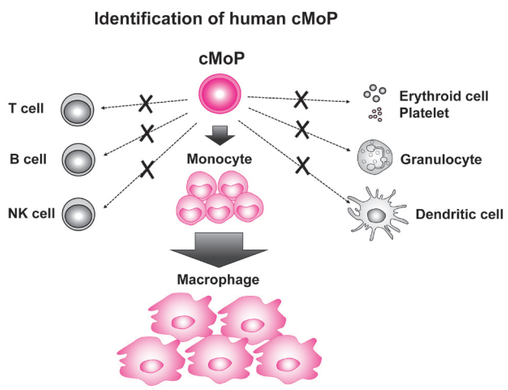


Fig.2 Human cMoP only gives rise to monocyte and macrophage

2) Roles of mononuclear phagocytes in inflammatory bowel disease

Breakdown of the intestinal epithelial layer's barrier function results in the inflow of commensal flora and improper immune responses against the commensal flora, leading to inflammatory bowel disease (IBD) development. Using a mouse dextran sodium sulfate (DSS)–induced colitis model, we showed that commensal Gram-positive bacteria trigger the mobilization of inflammatory monocytes and macrophages into the colon (***Mucosal Immunol*** 2015). TNF-α, a representative cytokine that aggravates colitis and a promising therapeutic target, was predominantly produced by monocytes/macrophages. Among macrophage subpopulations, Ly6c$^+$ macrophages were a major colitogenic subset producing TNF-α. In addition, IFN-γ–Stat1 pathway was required for histone acetylation at the promoter regions of the *Tnf* loci in macrophages, indicating that IFN-γ–dependent epigenetic regulation instructs the development of colitogenic macrophages. Our study may provide new therapeutic targets, e.g. inhibition of acetyl transferase in macrophage, for treating IBD and colon cancer (in revision).

2. Research on tissue stem cells

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

We found that type I IFNs induce proliferation and exhaustion in hematopoietic stem cells (HSCs), and that interferon regulatory factor-2 (IRF2), a transcriptional suppressor of type I IFN signaling, preserves the self-renewal and multi-lineage differentiation capacity of HSCs (***Nat Med*** 2009). Based on this finding, we show that type I IFN preconditioning, without irradiation or DNA alkylating agents, significantly enhanced the HSC engraftment efficiency in wild type (WT) recipient mice (***Blood*** 2013). Based on these achievements, we have further found that physiological levels of type I IFN signaling also affect other tissue stem cells, e.g. intestinal stem cells (ISCs) and hair follicle stem cells (HFSCs). Elucidation of detailed mechanisms is currently in progress.

3. Collaborative research with other institutes

In collaboration with RIKEN, Institute of Physical and Chemical Research, we performed microbiota analysis by 16S rRNA sequencing, and found that there is no significant change in the feces of mice with excess IFN signals specifically in intestinal epithelial cells. As the mice showed defective regeneration capacity of ISCs, we concluded that it is unlikely due to the altered commensal composition (manuscript in preparation).

Publications

[original papers]

1. Yokoi T, Yokoi K, Akiyama K, Higuchi T, Shimada Y, Kobayashi H, Sato T, Ohteki T, Otsu M, Nakauchi H, Ida H and Ohashi T. Non-myeloablative preconditioning with ACK2 (anti-c-Kit antibody) is efficient in bone marrow transplantation for murine models of mucopolysaccharidosis type II. ***Mol Genet Metab*** 119, 232-8 (2016).
2. Onai N, Ohteki T. Isolation of dendritic cell progenitor and bone marrow progenitor cells from mouse. ***Methods Mol Biol*** 1423, 53-9 (2016).
3. Liu J, Guo YM, Onai N, Ohyagi H, Hirokawa M, Takahashi N, Tagawa H, Ubukawa K, Kobayashi I, Tezuka H, Minamiya Y, Ohteki T and Sawada K. Cytosine-phosphorothionate- guanine oligodeoxynucleotides exacerbates hemophagocytosis by inducing tumor necrosis factor-α production in mice after bone marrow transplantation. ***Biol Blood Marrow Transplant*** 22, 627-36 (2016).

[Awards]

Shunsuke Kawamura (Research Support Assistant), Young Investigator Award of the 24th International Symposium on Molecular Cell Biology of Macrophages in 2016
Shunsuke Kawamura (Research Support Assistant), Young Investigator Award of Immunology and Pathological Biochemistry Fields Joint Symposium of TMDU in 2016

[Personnel changes]

Nobuyuki Onai, Professor, Department of Immunology, Kanazawa Medical University, Kanazawa, Japan.

Department of Bio-informational Pharmacology

Professor	Tetsushi Furukawa, M.D., Ph.D.
Associate Professor	Junko Kurokawa, Ph.D. (until October 2016)
	Jun Takeuchi, Ph. D. (since November 2016)
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. Pathogenesis of atrial fibrillation (AF)

Up to the present, we have carried 3 GWAS (genome-wide association study); 1st GWAS with 250K SNPs, 2nd GWAS with 610K SNPs, and 3rd GWAS with 1000K SNPs. The 1st GWAS gave us 2 AF-sensitive SNPs, the 2nd GWAS 13 AF-sensitive SNPs (Nat. Genet. 2012;44;670-675, Circulation 2014;130:1225-1235), and the 3rd GWAS 16 AF-sensitive SNPs (Nat. Genet. in press). Using the SNPs identified in the 2nd GWAS, we constructed weighed genetic risk score (GRS), which could predict AF development with 60% sensitivity and 61% specificity (Can. Heart J. 2017;33;443-449, J. Cardiol. 2017 [in press]).

2. Pannexin for ischemic preconditioning

Pannexin is a member of gap-junction channel, which is not involved in the formation of gap-junction channels between cells, but provides hemi-channels on the surface of the membrane. Pannexin functions as chloride channels in the basal condition and as ATP-release channels after mechanical stimuli. Being suffered from myocardial infarction, hearts with prior ischemic events (angina) develop reduced size infarcted size compared with those without prior ischemic events. The effect of prior ischemic events is referred as “ischemic pre-conditioning”. Though extracellular ATP is known to play an important role for ischemic pre-conditioning, the source of ATP is not known. Using pannexin-1 (a major isoform of pannexin in hearts) KO mouse, we found that ATP released during hypoxia was much lower in KO mice than in WT mice. Ischemic pre-conditioning reduced the infarcted area in WT mice, which was almost abolished in KO mice. Taken together, we conclude that hypoxia-activated pannexin provides extracellular ATP, which is required for the ischemic pre-conditioning.

3. Safety cardiac pharmacology and toxicology using iPS cells and mathematical modeling

We aim to contribute to assessments for drug-induced lethal arrhythmias which have been a major reason for drug withdrawal from market. Novel multidisciplinary approaches using in silico mathematical models (collaborating with Dr. Takashi Ashihara at Shiga University of Medical Science) and human iPS cells-derived cardiomyocytes (collaborating with Dr. Yasunari Kanda and Dr. Yuko Sekino at National Institute of Health Sciences) are developing in order to predict lethal drug-induced arrhythmias at pre-clinical safety pharmacological and toxicological tests. In this year, we developed a simulation model which can describe automaticity of electrical activity of human iPS cells-derived cardiomyocytes, and found that KCNJ2 expression is crucial for the automaticity (under revision).

Highlight

Cardiac development and regeneration

The technologies for cardiomyocyte induction from human iPSCs gave us one of progressive tools for clinical application. However, the cardiomyocyte characters greatly vary by their differentiation time and their places. Even long-cultured cardiomyocytes form human iPSCs still keep immature characters with arrhythmia or tachycardia (森田ら 2016). To address this issue, we identified that a novel transcription factor, Sall1 acts as a key player for heart induction via regulation of major cardiac genes such as *Islet1/Nkx2-5/Tbx5/Gata4/Mef2c* etc (Morita et al., ***JMCC*** 2016) (Fig. 1), and cooperatively functions with a cardiac gene for heart specification and its maturation (Morita et al., submitted 2017). We also found that these genes function during cardiac regeneration in mammals (Nakamura et al., ***Develop. Growth. Differ.*** 2016). In addition, to address the mechanisms of congenital heart defects and heart failure in adults, we found the evidence that some epigenetic genes from RNA-sequence between males and females in human/rodents might enhanced these symptoms more worse (Tsuji et al., revised 2017; Hori et al., submitted 2017).

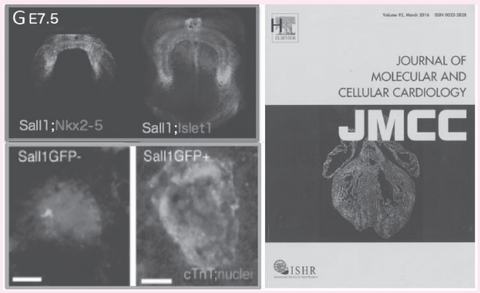


Fig.1 A novel cardiomyocyte progenitor cell: Sall1⁺ cell

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1. Yoshioka S, Usami T, Kikuta J, Ishii M, Sasano T, Sugiyama K, Furukawa T, Nakasho E, Takayanagi H, Tedder T, Karasuyama H, Kiyawami A, Adachi T. Intravital imaging of Ca^{2+} signals in lymphocytes of Ca^{2+} biosensor transgenic mice: indication of autoimmune diseases before the pathological onset. ***Sci. Rep.*** 2016:18738.
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5. Lopez-Redondo F, Kurokawa J, Nomura F, Kaneko T, Hamada T, Furukawa T, Yasuda K. A distribution analysis of action potential parameters obtained from patch-clamped human stem cell-derived cardiomyocytes. ***J. Pharmacol. Sci.*** 2016;131(2):141-145.
6. Hasegawa Y, Hamada S, Nishimura T, Sasaki T, Ebana Y, Kawabata M, Goya M, Isobe M, Koyama T, Furukawa T, Hirao K, Sasano T. Novel dielectric coagulation identifies hypercoagulability in patients with a high CHADS2 score without atrial fibrillation. ***PLoS One*** 2016;11:e0156557.
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Department of Stem Cell Regulation

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Assistant Professor	Kouichi TABU
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Research Outline

Our research has been conducted to elucidate the mechanisms by which stem cells are regulated. The major focus has been on neural stem cells, hematopoietic stem cells, and cancer stem cells. The study is aimed to understand development, maintenance, and regeneration of the central nervous system and the hematopoietic system, and to obtain a clue to tackle the problem of cancer recurrence. The projects performed in 2016 are categorized into three groups: 1. Understanding of molecular basis for self-renewal and fate determination of neural stem cells, 2. Characterization of fetal hematopoiesis, and 3. Characterization of cancer stem cells and their niche.

Research Projects

1. Epigenetic regulation of brain function

Physiological and pathological roles of histone modification, particularly histone methylation, in higher brain functions largely remained elusive. *Gasc1* gene encodes a histone H3 lysine 9 (H3K9) demethylating enzyme, which is considered to lead to a wide range of epigenetic activation of gene expression (Figure 1). We have been analyzing the mice with a hypomorphic mutation in the *Gasc1* gene in collaboration with Professor Inazawa at Medical Research Institute, Tokyo Medical and Dental University (TMDU), and found that *Gasc1* is strongly expressed in post-mitotic neurons in the embryonic and adult mouse forebrain. Although *Gasc1* hypomorphic mice showed no obvious histological abnormalities in the brain, they exhibited abnormal behaviors, including hyperactivity, stereotyped behaviors and impaired learning and memory. These symptoms appeared to be relevant to human neurodevelopmental disorders. In this year, we have focused on spine density in the hippocampal dendrites. We found that the spine density in the *Gasc1* hypomorphic mice was lower at 1 month of age and higher at 2.5 month. It was comparable to that in normal brain at 7.5 month. We further analyzed the density of morphologically mature spines in the hippocampus, and found that it was significantly higher in *Gasc1* hypomorphic mice at 2.5 month. These findings may help understand neurodevelopmental disorders.

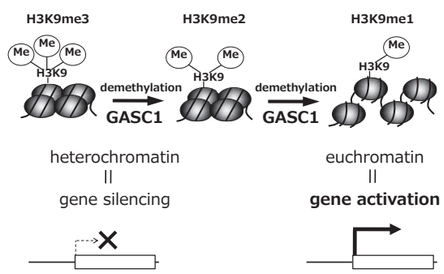

Figure 1: Gene activation by histone demethylase GASC1

2. Involvement of transcription factor Sox17-mediated expression of adhesion molecules in hematopoietic cell cluster formation in midgestation mouse dorsal aorta.

Definitive hematopoiesis is firstly detected in the aorta-gonad-mesonephros (AGM) region in midgestation mouse embryo and hematopoietic stem cells are contained within intra-aortic hematopoietic clusters (IAHCs) of dorsal aorta. A transcription factor Sox17 is expressed in the IAHCs and that Sox17 overexpression in CD45^{low}c-Kit^{high} cells comprising IAHCs in the co-culture with stromal cells maintained the formation of hematopoietic cell clusters in vitro. In such Sox17-transduced cells, vascular endothelial-cadherin (VE-cad) and endothelial cell-selective adhesion molecule (ESAM) were expressed at high levels. Here we show the involvement of VE-cad and ESAM in the formation of IAHCs (Figure 2). Sox17 was further shown to be involved in the expression of these adhesion molecules. We showed that VE-cad and ESAM were expressed in the vascular endothelial cells of dorsal aorta and in IAHCs. VE-cad and ESAM were also expressed in Sox17-transduced cells. Moreover, we observed that the VE-cad and ESAM expression levels in the Sox17-transduced cell clusters are higher as compared with those in the non-clustering hematopoietic colonies. The multipotent colony-forming activity in semisolid media was high in the VE-cad$^+$ cells and ESAM$^+$ cells among Sox17-transduced cells. Furthermore, Sox17 directly bound to the *Cdh5* (VE-cad) and *ESAM* promoters and induced their expression. shRNA-mediated knockdown of VE-cad or ESAM in Sox17-transduced cells decreased the number of hematopoietic clusters. These findings suggest that VE-cad and ESAM paly an important role in the hematopoietic cluster formation in midgestation mouse dorsal aorta.

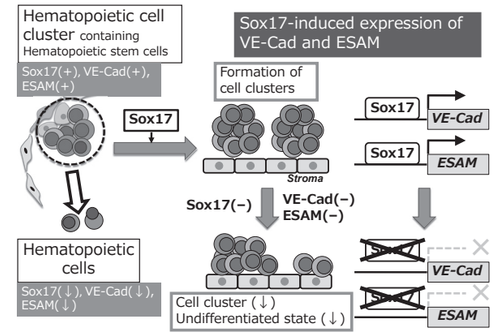

Figure 2: Involvement of Sox17-induced VE-Cad and ESAM in fetal hematopoiesis

3. Elucidation of metabolism and its application to cancer stem cell diagnosis

"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. As we published in 2004, C6 glioma cell line contains a subpopulation of CSCs, which is enriched in the "side population (SP)" by Hoechst 33342 staining and FACS analysis. In the recent couple of years, we aimed to elucidate metabolic status of CSCs and to develop the new methods to capture and eradicate CSCs. When stained with fluorescent probes for the assessment of mitochondrial amount, ROS levels etc., SP cells exhibits lower fluorescence. But some of tested probes were found to be excluded from SP cells through ABC transporters, indicating that careful interpretations must be required for the xenomaterials-based characterization of CSCs. Since transferrin receptor that mediates cellular uptake of iron was identified as a gene upregulated in SP cells, we next focused on iron-related metabolic pathways in CSCs, especially on protoporphyrin IX (PpIX)-heme biosynthesis pathway. PpIX is an intermediate substance metabolized from an endogenous amino acid "5-aminolevulinic acid" (5-ALA) and converted into heme through the insertion of ferrous iron. Given that PpIX preferentially accumulates in tumor cells and has a photosensitizing property, 5-ALA is commonly given for fluorescence-guided surgical resection of malignant gliomas. However, it has not been fully examined whether this method could be effective for CSC detection. When C6 cells were treated with 5-ALA, SP cells exhibits lower accumulation of PpIX fluorescence, suggesting that CSCs may escape from 5-ALA-based photodynamic detection and surgical resection (Figure 3, upper panel). Expectedly, the poorer accumulation was found to be improved by the combined use of 5-ALA with an iron chelator deferoxamine (DFO), indicating that CSCs have increased consumption of iron in converting PpIX into heme. These data will provide clues to develop effective diagnostic/therapeutic strategies for cancer eradication (Figure 3, lower panel).

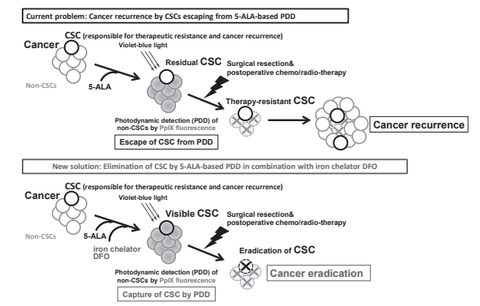

Figure 3: A current limitation and a new strategy to detect CSCs for cancer eradication

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[Original Article]

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6. Murota Y, Tabu K, and Taga T. Requirement of ABC transporter inhibition and Hoechst 33342 dye deprivation for the assessment of side population-defined C6 glioma stem cell metabolism using fluorescent probes. BMC Cancer, 16:847, 2016
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Department of Structural Biology

Professor Nobutoshi Ito
Associate Professor Teikichi Ikura
Assistant Professor Nobutaka Numoto

The advance of genome science and proteomic analysis has produced a large amount of information about the primary structure of proteins and their spatial and temporal distributions. On the other hand, most of the proteins only function when they take certain three dimensional structures. As obviously seen in so-called prion diseases, proteins which are chemically correct but structurally incorrect not only fail to function properly but also can harm cells. Our laboratory aims to understand the function of biological macromolecules at atomic level through structure analysis and other methods of physical chemistry, in the hope that accumulation of such knowledge will eventually lead to development of drugs. We are also involved in providing database of such structural data to scientists through the activities of Protein Data Bank Japan.

1. Structural and functional analysis of the B-cell inhibitory co-receptor CD72

One of the causes of autoimmune diseases is excessive response of B cells and production of autoantibodies. CD72 is primarily expressed in B cells and negatively regulates B cell antigen receptor signaling. We have determined the crystal structure of the ligand-binding domain (C-type lectin-like domain) of CD72 at 1.2 Å resolution, using the synchrotron radiation facilities (Akatsu et al., J. Exp. Med., 2016). We have also demonstrated that CD72 specifically binds to Sm/RNP, an RNA-containing nuclear self-antigen, and inhibit B cell response, preventing production of anti-Sm/RNP antibody crucial for development of systemic lupus erythematosus (SLE).

Analysis of the electrostatic potentials of CD72[a], one of the three characterized mouse CD72 alleles which we determined the crystal structure in this study, reveals that there is a highly positively charged patch on the surface of the molecule (Fig. 1). Because the molecules with nucleic acids such as Sm/RNP possesses negatively charged region derived from the sugar-phosphate backbone, it is strongly suggested that the positively charged patch of CD72[a] is related to recognition of the nucleic acids in Sm/RNP. In addition, we calculated the homology model of CD72[c], which is an allele of CD72 in a model mouse of an autoimmune disease. The model clearly shows that surface charge distribution around the putative Sm/RNP binding region is different from that of CD72[a], substitutions of some residues in this area result in generation of a negative-charge patch. It would be disadvantageous for binding Sm/RNP due to electrostatic repulsion with the negative charge of RNA. In fact, binding affinity to Sm/RNP is decreased in CD72[c], which is considered to be triggering autoimmune disease. Therefore, the charge distribution on the molecular surface of CD72 is thought to play an important role in controlling the recognition and binding of Sm/RNP.

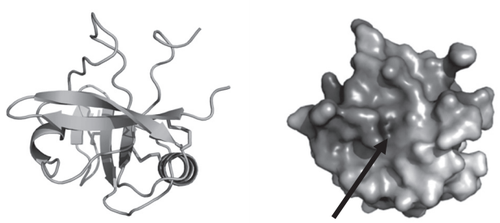

Fig.1 Crystal structure of the ligand-binding domain of CD72[a], represented as ribbon diagram (left) and surface model (right). The positive charge patch is indicated by the arrow.

Based on these results, in order to elucidate the more detailed molecular mechanism of ligand binding of CD72, we are advancing the crystal structure analysis of the ligand-binding domain of CD72[c]. It is expected that very useful knowledge will be obtained for rational design of new molecules that control CD72 function, to establish a new treatment method for autoimmune diseases using the function of CD72. This work is performed in collaboration with Professor Tsubata of Department of Immunology.

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

Huntington's disease is an inherited disease that causes the progressive degeneration of nerve cells in the brain, in which mutant Huntingtin (Htt) proteins abnormally interact with an essential DNA damage repair protein Ku70 in neurons and degenerate its function. In this study, we explored new drug seeds to interrupt the abnormal interactions between them. Finally, we succeeded in discovering three small chemicals with therapeutic effects. According to the dynamic light scattering analysis (Fig. 2), these chemicals most likely interact with mutant Htt and change its dynamics and aggregative propensity. This work is performed in collaboration mainly with Professor Okazawa in our institute, and with several research groups at Tohoku University, Keio University, National Institute of Advanced Industrial Science and Technology, and Gladstone Institute.

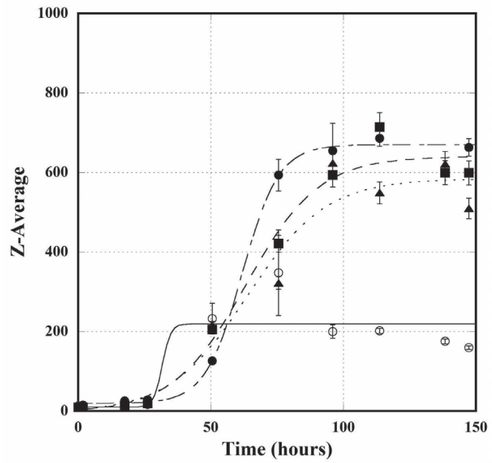

Fig.2 The three chemicals, 7H (filled circle), Ang III (filled square), and LH-RH fragment (filled triangle), affect the dynamic light scattering measurements of a mutant Htt aggregation (open circle) in vitro.

3. Improvement in Protein Data Bank

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

Publications

1. Isao Kii, Yuto Sumida, Toshiyasu Goto, Rie Sonamoto, Yukiko Okuno, Suguru Yoshida, Tomoe Kato-Sumida, Yuka Koike, Minako Abe, Yosuke Nonaka, Teikichi Ikura, Nobutoshi Ito, Hiroshi Shibuya, Takamitsu Hosoya, Masatoshi Hagiwara. Selective inhibition of the kinase DYRK1A by targeting its folding process. Nat Commun. 2016; 7; 11391
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Frontier Research Unit
Laboratory of Oxygen Biology

Associate Professor Koh Nakayama, Ph.D.
Assistant Professor Ryo Yonashiro, Ph.D.

Aim of the Research

Study in our laboratory aims to understand how changes in the ambient oxygen concentration affect our body function. Oxygen is required for the efficient energy production in mitochondria. Furthermore, recent studies revealed the role of oxygen, particularly a hypoxic condition, involved in developmental processes, tumorigenesis, and stem cell function. Our goal is to understand the molecular mechanism of hypoxic response, and establish new tools for cancer therapy and regenerative medicine.

Research Projects

1. Metabolic regulation under hypoxic condition

Hypoxia-Inducible Factor (HIF)-α is a transcription factor which plays a central role during hypoxic response. HIF-α is actively degraded during normoxic condition, whereas it is stabilized and activated under hypoxic conditions. PHD hydroxylates and regulates the expression of HIF-α. There are three PHDs in mammals; namely PHD1, 2, and 3. We have previously identified that PHD3 forms a complex under hypoxic condition (Figure). This complex serves to stabilize HIF-α by inhibiting the access of PHD3 to HIF-α.

Moreover, we recently identified that pyruvate dehydrogenase (PDH) is included in this complex (Figure). PDH is an enzyme which converts pyruvate into acetyl CoA, and plays a key role in energy metabolism. We have demonstrated that PDH interacts with PHD3 under hypoxic condition. In *PHD3*$^{-/-}$ cells, PDH activity is significantly decreased, suggesting that PHD3 positively regulates PDH activity by directly interacting with it. Cancer cells are known to exhibit glycolytic metabolism; however, the molecular mechanism of how such metabolism is formed has not been elucidated yet. We aim to understand the mechanism by focusing on the PHD3-PDH interaction mediated by the hypoxic complex.

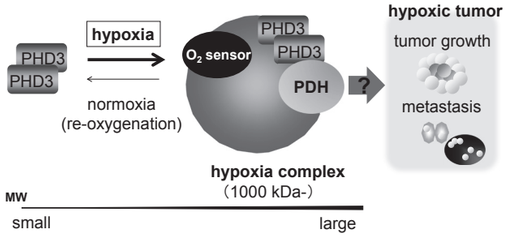

Figure Cellular metabolism regulated by Hypoxia Complex

2. Chronic hypoxic response regulated by a crosstalk of CREB and ER stress response pathways

We have previously demonstrated that the expression and activity of HIF decreases and that CREB and NF-κB are activated during chronic phase of hypoxia. We now focus on CREB to further elucidate the signaling machinery during chronic hypoxia. Recently, we identified that ER stress response pathway is activated during chronic hypoxia and cross-talks with CREB. Knockdown of CREB in breast cancer cells reduced the expression of two ER stress responsive molecules, PERK and IRE1α, which led to decreased ER stress response in these cells. Consequently, CREB-depleted cells exhibited less tumor metastasis in a tumor mouse model. This study highlights the possibility of CREB to be a therapeutic target for cancer.

Publications

1. Kikuchi D., Tanimoto K., and **Nakayama K.*** CREB is activated by ER stress and modulates the unfolded protein response by regulating the expression of IRE1α and PERK. ***Biochem. Biophys. Res. Commun.*** 469, 243-250, (**2016**).
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Tenure Track Research Unit
Department of Cellular and Molecular Medicine

Associate professor Yumiko Oishi MD, PhD
Assistant professor Shinichiro Hayashi, PhD, Sumio Hayakawa, PhD
Research Technician Yukiko Hoshino, Yumi Suzuki

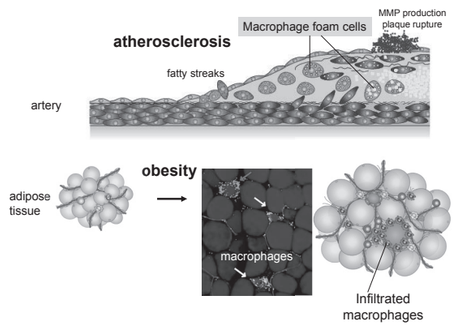
Research outline

Cardiovascular disease, as a consequent of the obesity related metabolic syndrome, remains a significant cause of morbidity and mortality in industrialized societies. A major effort of our laboratory has been to investigate the molecular mechanism of metabolic syndrome from the viewpoint of transcriptional regulation. We focus on macrophage and skeletal muscle as major players in those context. The long term goals of our current study are to elucidate: 1) the mechanism of the link between cellular metabolism and immune response in macrophage 2) the mechanism of chronic inflammation leads to metabolic syndrome, and 3) the mechanism responsible for pathogenesis of sarcopenia and skeletal muscle degeneration.

Research Project

1. Mechanisms of Coordinated regulation of inflammatory response and lipid homeostasis in macrophage

Chronic low-grade inflammation has been recognized as a key contributing factor in the onset and progression of metabolic syndrome and atherosclerosis. As a multifunctional effector cell, macrophage play pivotal roles in both the enhancement and resolution of this inflammatory process (Figure). By utilizing molecular biology technique, lipidomics and bioinformatics, we found that the lipid homeostasis is coordinately regulated with inflammatory response in macrophage. TLR4 activation rapidly, and transiently inhibits Liver X receptor (LXR) signaling, and subsequently activates Sterol regulatory element-binding protein (SREBP). In the late phase of inflammation, LXR and SREBP work together to increase anti-inflammatory fatty acid synthesis, necessary for a resolution of inflammation. Thus, transcriptional/signaling network involving LXR and SREBP play a pivotal role in the regulation of lipid homeostasis and cellular function. By elucidating the crosstalk between cellar function and metabolism, we would be able to accumulate beneficial knowledge to develop novel therapeutic strategy targeting macrophages for the prevention and treatment of metabolic syndrome.



Macrophage is important for a chronic inflammation.

2. Mechanism of skeletal muscle degeneration

Skeletal muscle consume ~40% of total energy, playing a key role for the pathogenesis of metabolic syndrome. Sarcopenia is the degenerative loss of skeletal muscle mass, quality and strength associated with aging. Although the causes and mechanisms of sarcopenia still remains unclear, one of the hypotheses is dysfunction of satellite cells, muscle stem cells in muscle. We identified KLF5 as a novel factor that play pivotal roles in skeletal muscle degeneration. Klf5 is a Zinc-finger transcription factor involved in the self-renewal and proliferation of embryonic stem cell and cancer stem cell. KLF5 is transiently induced in the myogenesis, plays critical role for muscle degeneration and repair. Although Klf5 is not expressed in the quiescent satellite cells, its expression is dramatically increased in the satellite cells with age. Now we are testing the hypothesis whether the dysregulation of Klf5 causes a mulfunction of satellite cells.

Publications

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Frontier Laboratory: Skeletal Molecular Pharmacology

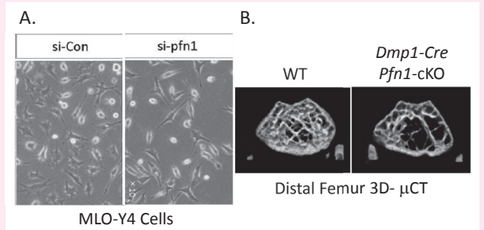
Associate Professor　Yoichi Ezura, MD, PhD

Summary

This laboratory aims to elucidate the molecular regulation of the cellular responses in organs and tissues involved in the calcium regulatory system. We focus on obtaining knowledge contributing to the establishment of the prevention and treatment of skeletal rare diseases and common diseases including osteoporosis.

Highlight

Profilin1 in osteocytes regulates cell shape, movements and bone mass (Lin Wanting et al) .
Osteocytes, the most abundant cells in bone, regulates bone metabolism in coordination with osteoblasts and osteoclasts. Their characteristic dendritic process based on actin cytoskeleton would be important. Assuming the possible involvement of an actin-binding protein involved in actin polymerization, Profilin1, we investigated its expression and function in osteocytes. Profilin1 mRNA was expressed in osteocytic MLO-Y4 cells and its levels were gradually increased along with the time in culture. With regard to functional aspect, Profilin1 knockdown by siRNA enhanced BMP-induced increase in alkaline phosphatase expression in MLO-Y4 cells. Profilin1 knockdown also suppressed the levels of dendritic processes and migration of MLO-Y4 cells. As possible contributor for the ageing related bone loss, hydrogen peroxide treatment increased the levels of profilin1 mRNA in MLO-Y4 cells in contrast to the decline in alkaline phosphatase. Profilin1 was expressed not only in MLO-Y4 cells but also in the primary cultures of osteocytes, and its mRNA levels were higher than those in primary osteoblasts in culture. To examine in vivo role of profilin1 in osteocytes, proflin1 was conditionally knocked out by using DMP1-cre and profilin1 floxed mice. This conditional deletion of profilin1 specifically in osteocytes resulted in reduction in the levels of bone volume and bone mineral density. These results indicate that Profilin1 expressed in osteocytes regulates cell shape, migration and bone mass. (***J Cell Physiol***, in press)



Profilin1 is essential for osteocyte dendrites and bone mass maintenance.
(A)Profilin1 knockdown in cultured MLO-Y4 impaired the dendrite formation.
(B)The osteocyte specific conditional Prfilin1 knock-out resulted in significant bone loss in adult femur.

Publications

[Original articles]

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2: Izu Y, Ezura Y, Koch M, Birk DE, Noda M. Collagens VI and XII form complexes mediating osteoblast interactions during osteogenesis. ***Cell Tissue Res.*** 2016 Jun;364(3):623-35.
3: Moriya S, Izu Y, Arayal S, Kawasaki M, Hata K, Pawaputanon Na Mahasarakhahm C, Izumi Y, Saftig P, Kaneko K, Noda M, Ezura Y. Cathepsin K Deficiency Suppresses Disuse-Induced Bone Loss. ***J Cell Physiol.*** 2016 May;231(5):1163-70.
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5: Pawaputanon Na Mahasarakham C, Ezura Y, Kawasaki M, Smriti A, Moriya S, Yamada T, Izu Y, Nifuji A, Nishimori K, Izumi Y, Noda M. BMP-2 Enhances Lgr4 Gene Expression in Osteoblastic Cells. ***J Cell Physiol.*** 2016 Apr;231(4):887-95.
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7: Katsumura S, Ezura Y, Izu Y, Shirakawa J, Miyawaki A, Harada K, Noda M. Beta Adrenergic Receptor Stimulation Suppresses Cell Migration in Association with Cell Cycle Transition in Osteoblasts-Live Imaging Analyses Based on FUCCI System. ***J Cell Physiol.*** 2016 Feb;231(2):496-504.
8: Lin W, Izu Y, Smriti A, Kawasaki M, Pawaputanon C, Böttcher RT, Costell M, Moriyama K, Noda M, Ezura Y. Profilin1 Is Expressed in Osteocytes and Regulates Cell Shape and Migration. ***J Cell Physiol.*** [in press]
9: Katsumura S, Izu Y, Yamada T, Griendling K, Harada K, Noda M, Ezura Y. FGF Suppresses Poldip2 Expression in Osteoblasts. ***J Cell Biochem.*** [in press]
10: Pawaputanon Na Mahasarakham C, Izu Y, Nishimori K, Izumi Y, Noda M, Ezura Y. Lgr4 Expression in Osteoblastic Cells Is Suppressed by Hydrogen Peroxide Treatment. ***J Cell Physiol.*** [in press]
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Division of Pathophysiology

【Aim and Scope】

Research purpose of the Division of Pathophysiology is to understand the fundamental mechanisms underlying biological phenomena and their abnormalities in the disease conditions including intractable diseases and to develop new diagnostic or therapeutic tools for various diseases of which curative treatment is difficult. Topics of research projects in each Department are as follows;

【Neuropathology】

- Development of a new antibody therapy against Alzheimer's disease.
- Discovery of a novel type of necrosis in Huntington's disease.

【Pathological Cell Biology】

- Identification of Cep63 as an autophagy-dependent regulator of centrosome number.
- Discovery of Golgi membrane-associated protein degradation pathway (GOMED) in yeast and mammals.

【Developmental and Regenerative Biology】

- How cell populations weed out "Loser" cells to maintain vitality is revealed
- Metabolic pathway regulating key stage of embryo development revealed

【Stem Cell Biology】

- Identification of stem cells in the skin
- Mechanisms of skin and hair aging

【Immunology】

- Elucidation of a novel mechanism that distinguishes self-nucleic acids from microbial nucleic acids and inhibits development of systemic lupus erythematosus.
- Establishment of the method to identify novel molecules that regulate the inhibitory B cell co-receptor CD22/Siglec-2 in a glycan-dependent manner.

【Molecular Pathogenesis】

- Diversity of MHC class I genes is the most useful genetic marker to clarify the diversification of species in Penguins.
- Loss-of function variations in Lp-PLA2 are not risk factors for cardiovascular diseases, explaining the failure in clinical trial of Lp-PLA2 inhibitor for cardiovascular events.

Department of Neuropathology

Professor	Hitoshi Okazawa
Practical professor	Kazuhiko Tagawa
Project Lecturer/Part-time Lecturer	Haruhisa Inoue, Masaki Sone, Toshiki Uchihara
Project assistant lecturer	Hiroshi Tsuda
Assistant professor	Kyota Fujita
Project Assistant professor	Xigui Chen, Hidenori Homma, Kazumi Motoki, Emiko Yamanishi

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. Strategy of molecular target for pathological signaling before aggregation of beta-Amyloid.

Pathological feature of neurodegenerative disease such as Alzheimer's disease is abnormal protein aggregation inside or outside of cells. In Alzheimer's disease, senile plaque composed of beta-Amyoid and neurofibrillary tangle composed of tau. Based on amyloid hypothesis, clinical trials have been challenged with the aim of clearance of extracellular amyloid aggregation. However, antibody therapy against beta-Amyloid did not succeed the recovery of cognitive decline although it could reduce the amyloid plaque. Therefore, our recent research interest is focusing on the earlier interpretation of antibody treatment before aggregation of beta-Amyloid, or new molecular target therapy based on early-phase (phase 0) change of pathological signaling.

Previously we performed comprehensive phosphoproteome analysis in order to identify the phase 0 molecular events, and we identified 17 core-protein phosphorylation changed from early-phase to late-phase, and among them, 3 phosphoprotein were changed at phase 0. MARCKS, one of these proteins, is known as a substrate of PKC, was phosphorylated not only PKC-phosphorylation site but also other phosphorylation site modified by another kinases. Thus, in the present study, we compared the change of molecular pattern in model mice and human postmortem brain in order to detect the key phosphorylation site. We found that Ser46 were phosphorylated at early-phase, and antibody against pSer46-MARCKS depicted degenerative neuritis surrounded the amyloid plaques. We also found the pSer46-MARCKS lost its ability to bind cytoskeletal protein, actin, and failed to maintain the spine. Phosphorylation of MARCKS at ser46 was due to HMGB1 (one of the DAMPS; damage-associated molecular pattern), not beta-Amyloid. Also, HMGB1 in spinal fluid of patients with early progression was shown higher than others, which suggest that pSer46-MARCKS closely related the pathology of Alzheimer's disease in human patients. Finally, we tried the rescue experiments using neutralizing antibody against HMGB1, and we succeeded the decrease of pSer46-MARCKS, rescued the reduction of spine, and improved the cognitive decline in model mice. HMGB1 can be released from not only leakage from dead cells but also hyperactive living neurons. Therefore, antibody therapy targeting HMGB1 have a potential to pathology of phase 0 before aggregation of beta-amyloid, and it might be beneficial for prevention of AD pathogenenesis.

2. New therapeutic strategy for Huntington's disease: targeting the third atypical cell death.

The common important feature of neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, Spinocerebellar Ataxia, Amyotrophic lateral sclerosis, is slow-progressive neurodegeneration over the decades. Such slow-progressive dysfunction of neuronal cells and their cell death have different aspects from other neurological disorders such as cerebral infarction or cerebral hemorrhage, which symptoms have been completed within a few minutes or a few hours. The characteristics of cell death in neurodegenerative disease have been greatly debated. The typical types of cell death are apoptosis and necrosis, but there are other types of cell death such as autophagy, necroptosis, and TRIAD (transcriptional repression-induced atypical cell death). We previously identified TRIAD, which can be triggered by specific inhibition of RNA polymerase II and can be executed slow-progressively. The morphological characteristics of TRIAD are different from that of apoptosis or autophagy, but key morphology is abnormal expansion of endoplasmic reticulum (ER). Also, we previously reported that YAP, a candidate molecule obtained through comprehensive expression analysis, might be involved in signal cascade of TRIAD execution. In the present studies, we first performed screening of TRIAD-related molecules associated with several types of cell death, then mixed with bioinformatics by matching the data with protein-protein interaction database in order to comprehensive search for identification of signal network of TRIAD. Then, we found hnRNP, a RNA-binding molecule, and Htt (causative gene for Huntington's disease) are related to TRIAD (Mao et al., 2016a)

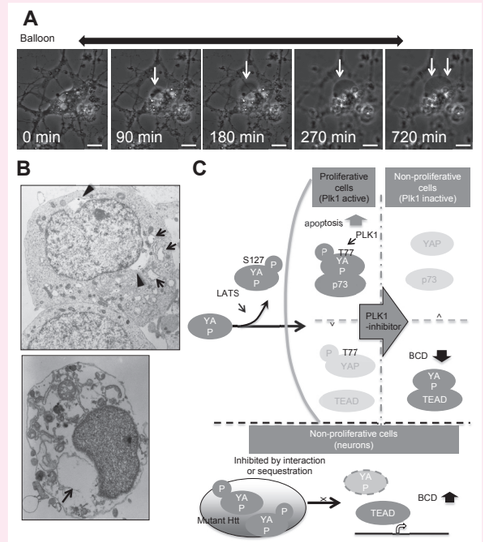
On the other hand, we found that mutant-Htt-expressing neurons showed not apoptotic or necrotic cell death but cell death with asymmetric cytoplasmic expansion. This expansion was endoplasmic reticulum expansion, and by two-photon microscopic analysis, we revealed that similar expansion of ER could be seen in living model mice brain carrying mutant Htt (Mao et al., 2016b).

We also tried to understand the molecular mechanism under the execution of TRIAD, and we found that loss of YAP-TEAD dependent survival signal is mainly contributed. Especially activation of Hippo-pathway prevented the intranuclear translocation of YAP, and phosphorylation of YAP by Plk1 increased binding of YAP/p73, also mutant Htt bounded to and incorporated YAP into its inclusion, which all leads to the loss of survival signaling (figure C in "Highlight"). Finally, we treated S1P to mutant Htt Knockin mice in order to promote YAP translocation to nucleus and prevent Hippo-pathway activation, and we succeeded to improve their behavioral impairment and the abnormal control of ER size. What is more, we found ER expansion in human HD patients by electron microscopy. We also confirmed the biochemical and histological alteration of hippo-pathway in human HD patients as well as HD model mice（Yamanishi et al., 2017）. Collectively, our present studies suggest that treatment targeting signaling pathway of TRIAD is beneficial for HD pathology.

Highlight

TRIAD, a third type of cell death, and neurodegenerative disease

We previously reported atypical cell death whose characteristics were different from apoptotic or autophagic cell death. TRIAD, transcriptional repression-induced atypical cell death, was identified by slow-progressive cell death caused by alpha-amanitin, a specific inhibitor of RNA polymerase II. By ultrastructual analysis using electron microscopy, such cells possessed abnormal expansion of ER. RNA polymerase II is necessary for basic transcription machinery in order to transcript mRNA from genomic DNA. In the research of neurodegenerative diseases, functional deficiency of nuclei, especially deficiency of transcription, has been reported over the decades. We have instantaneously anticipated polyglutamine tract sequence might be involved in nuclear transcription impairment, and we have previously reported such pathology; for example, identification of PQBP1 (polyglutamine binding protein 1). Based on such background research, we identified TRIAD, cell death caused by transcriptional repression. In the present studies, we found that mutant Htt expressing neurons showed balloon-like cytoplasmic expansion (figure A). We also found ER expansion by electron microscopy or labeling with organelle markers, which is the same morphological feature of TRIAD (figure B). Previously, in HD model mice, there were several reports indicating atypical cytoplasmic expansion. We revealed the induction of TRIAD by causative genes of neurodegenerative disease, and we determined molecular signaling of TRIAD induced by mutant Htt, which might make progress in understanding of relationship between TRIAD and neurodegenerative diseases (figure C).



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Department of Pathological Cell Biology

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

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

〈**Research Projects**〉

1, Analysis of Atg5/Atg7-independent alternative macroautophagy.

ATG5 and ATG7 are considered to be essential molecules for the induction of autophagy. However, we found that cells lacking ATG5 or ATG7 can still form autophagosomes/autolysosomes and perform autophagic protein degradation when subjected to certain types of stress. Although the lipidation of LC3 is accepted as a good indicator of autophagy, this did not occur during ATG5/ATG7-independent alternative autophagy. Unlike conventional autophagy, autophagosomes appeared to be generated in a Rab9-dependent manner by the fusion of the phagophores with vesicles derived from the *trans*-Golgi and late endosomes. Therefore, mammalian autophagy can occur via at least two different pathways; the ATG5/ATG7-dependent conventional pathway and an ATG5/ATG7-independent alternative pathway.

In this year, we discovered three novel findings on the conventional autophagy. First, autophagy controls centrosome number by degrading Cep63. The ubiquitin–proteasome system has been considered to be the main regulator of centrosome number. However, we showed that autophagy also regulates the number of centrosomes. Furthermore, we discovered that this autophagic regulation of centrosome number is dependent on a degradation of Cep63 via p62 interaction (Figure1). Second, autophagy has a role to control cell migration by degrading GEF-H1, a member of the RhoA family of GEF. Third, autophagy suppresses apoptosis induced by X-ray irradiation. This suppression was caused by the degradation of the proapoptotic molecule Noxa. In this paper, we also showed that genotoxic stress-induced autophagy is induced by the dephosphorylation of Ulk1 at Ser^{637}, and PPM1D is responsible for this dephosphorylation.

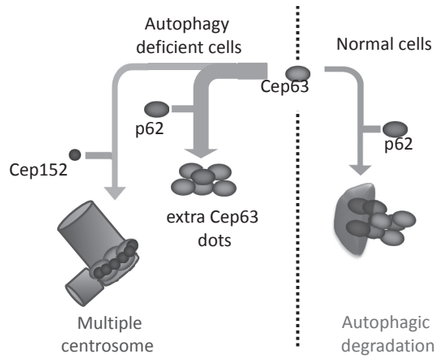

Figure1. Autophagy controls centrosome number by degrading Cep63.
Autophagy regulates the number of centrosomes, so that autophagy-deficient cells carry extra centrosomes. The autophagic regulation of centrosome number is dependent on a Cep63 given that cells lacking autophagy contain multiple Cep63 dots that are engulfed and digested by autophagy in wild-type cells, and that the upregulation of Cep63 increases centrosome number. Cep63 is recruited to autophagosomes via interaction with p62, a molecule crucial for selective autophagy.

In addition, we found the mechanism of alternative autophagy. When PI(4)P-dependent anterograde trafficking from the Golgi is disturbed, alternative autophagy is generated from the trans-Golgi membrane and degrades accumulated Golgi proteins. This machinery is phylogenetically conserved from yeast to mammals. We named this machinery Golgi-mediated degradation pathway (GOMED) (Figure2).

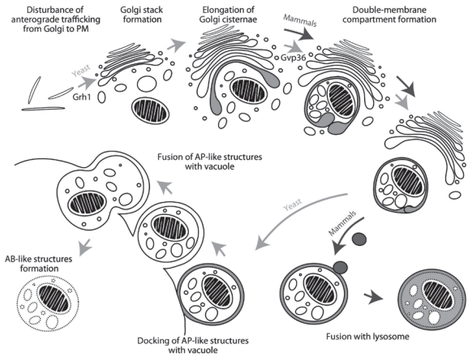

Figure2. Induction of a Golgi membrane-associated degradation pathway by the disturbance of anterograde trafficking from the Golgi
Novel Golgi membrane-associated degradation pathway is activated when PI(4)P-dependent Golgi-to-plasma membrane (PM) trafficking is disrupted in yeast. This machinery is a type of alternative autophagy. We named this pathway Golgi membrane-associated degradation (GOMED). The GOMED pathway also functions in Atg5-deficient mammalian cells when Golgi-to-PM trafficking is disrupted. In autophagy-deficient β-cells, the GOMED pathway functions to suppress insulin secretion by degrading unused insulin granules.

2, Molecular mechanisms of programmed cell death

Cell death is the essential mechanisms for selective elimination of cells. It plays an integral part in a variety of biological events, including morphogenesis and removal of harmful cells. Previously, apoptosis is considered as the only form of programmed cell death. Recent studies, however, have provided evidence that there is another mechanism of programmed cell death called non-apoptotic cell death, including include apoptosis, autophagic cell death, and programmed necrosis.

Apoptosis is induced via increases in mitochondrial membrane permeability, called mitochondrial outer membrane permeabilization (MOMP). In this year, we quantitatively characterized the Bak, a component of MOMP, *in situ* based on single molecule localization. We observed individual Bak proteins in the cluster with a precision of 20 nm and found that Bak proteins form densely-packed clusters at the nanoscale on mitochondria during apoptosis. These results suggest that the density of Bak proteins is a representative profile to characterize the cluster responsible for apoptosis.

3, Analysis of mitochondrial diseases

Mitochondria play an essential role in both cell survival and cell death. In both cell function, mitochondrial membrane permeability is crucial. Therefore, we are focusing on channel proteins located on mitochondrial membrane. We are also focusing on mitochondrial diseases.

List of Publications

[Original paper]

1. Autophagy suppresses cell migration by degrading GEF-H1, a RhoA GEF. *T. Yoshida*, *M. Tsujioka*, *S. Honda*, *S. Shimizu* **OncoTarget** 7:34420-9, 2016
2, In Situ Characterization of Bak Clusters Responsible for Cell Death Using Single Molecule Localization Microscopy. *Y. Nasu*, *A. Benke*, *S. Arakawa*, *G. Yoshida*, *G. Kawamura*, *S. Manley*, *S. Shimizu*, *T. Ozawa* **Scientific Reports** 6, Article number: 27505, 2016
3, Golgi membrane-associated degradation pathway in yeast and mammals. *H. Yamaguchi*, *S. Arakawa*, *T. Kanaseki*, *T. Miyatsuka*, *Y. Fujitani*, *H. Watada*, *Y. Tsujimoto*, *S. Shimizu* **EMBO J** 35:1991-2007, 2016
4, TRF2 Interacts with Core Histones to Stabilize Chromosome Ends. *A. Konishi*, *T. Izumi*, *S. Shimizu* **J. Biol. Chem.** 291(39):20798-810, 2016
5, Identification of PPM1D as an essential Ulk1 phosphatase for genotoxic stress-induced autophagy. *S. Torii*, *T. Yoshida*, *S. Arakawa*, *S. Honda*, *A. Nakanishi*, *S. Shimizu* **EMBO R** 11:1552-1564, 2016
6, Autophagy controls centrosome number by degrading Cep63. *Y. Watanabe*, *S. Honda*, *A. Konishi*, *S. Arakawa*, *M. Murohashi*, *H. Yamaguchi*, *S. Torii*, *M. Tanabe*, *S. Tanaka*, *E. Warabi*, *S. Shimizu* **Nature Commun** 7:13508, 2016
7, Mitochondrial damage elicits a TCDD-inducible poly(ADP-ribose) polymerase-mediated antiviral response. *T. Kozaki*, *J. Komano*, *D. Kanbayashi*, *M. Takahama*, *T. Misawa*, *T. Satoh*, *O. Takeuchi*, *T. Kawai*, *S. Shimizu*, *Y. Matsuura*, *S. Akira*, *T. Saitoh* **PNAS** *in press*

Department of Developmental and Regenerative Biology

Professor Hiroshi Nishina, Ph.D.
Associate Professor Jun Hirayama, Ph. D.
Assistant Professor Norio Miyamura, Ph.D.

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

1. SAPK/JNK Signaling Pathway

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

2. Hippo Signaling Pathway

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

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

3. Circadian Clock

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

Highlight

Cell competition is a cell-cell interaction by which a cell compares its fitness to that of neighboring cells. The cell with the relatively lower fitness level is the “loser” and actively eliminated, while the cell with the relatively higher fitness level is the “winner” and survives. Recent studies have shown that cells with high Yes-associated protein (YAP) activity win cell competitions but the mechanism is unknown. Here, we report the unexpected finding that cells overexpressing constitutively active YAP undergo apical extrusion and are losers, rather than winners, in competitions with normal mammalian epithelial cells. Inhibitors of metabolism-related proteins such as phosphoinositide-3-kinase (PI3K), mammalian target of rapamycin (mTOR), or p70S6 kinase (p70S6K) suppressed this apical extrusion, as did knockdown of vimentin or filamin in neighboring cells. Interestingly, YAP-overexpressing cells switched from losers to winners when co-cultured with cells expressing K-Ras (G12V) or v-Src. Thus, the role of YAP in deciding cell competitions depends on metabolic factors and the status of neighboring cells.

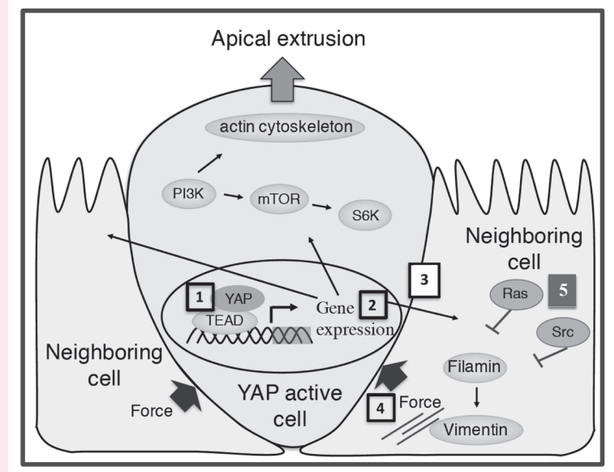

Fig.1. Schematic illustration of the proposed molecular mechanism of apical extrusion of active YAP-expressing MDCK cell.

Publications

1. Yoshimi Okamoto-Uchida, Ruoxing Yu, Norio Miyamura, Norie Arima, Mari Ishigami-Yuasa, Hiroyuki Kagechika, Suguru Yoshida, Takamitsu Hosoya, Makiko Nawa, Takeshi Kasama, Yoichi Asaoka, Reiner Wimmer Alois, Ulrich Elling, Josef M. Penninger, Sachiko Nishina, Noriyuki Azuma and Hiroshi Nishina (2016) The mevalonate pathway regulates primitive streak formation via protein farnesylation. ***Scientific Reports*** 6, 37697. Press release
2. Takanori Chiba[1], Erika Ishihara[1], Norio Miyamura, Rika Narumi, Mihoko Kajita, Yasuyuki Fujita, Akira Suzuki, Yoshihiro Ogawa and Hiroshi Nishina (2016) Active form of YAP expressing MDCK cells are extruded apically depending on neighboring cells status. ***Scientific Reports*** 6, 28383. Press release
3. Yoichi Asaoka, Yoko Nagai, Misako Namae, Makoto Furutani-Seiki and Hiroshi Nishina (2016) SLC7 family transporters control the establishment of left-right asymmetry during organogenesis in medaka by activating mTOR signaling. ***Biochem. Biophys. Res. Commun.*** 474, 146-153.
4. Miki Nishio, Keishi Sugimachi, Hiroki Goto, Jia Wang, Takumi Morikawa, Yosuke Miyachi , Yusuke Takano, Hiroki Hikasa, Tohru Itoh, Satoshi O Suzuki, Hiroki Kurihara, Shinichi Aishima, Andrew Leask, Takehiko Sasaki, Toru Nakano, Hiroshi Nishina, Yuji Nishikawa, Yoshitaka Sekido, Kazuwa Nakao, Kazuo Shin-ya, Koshi Mimori and Akira Suzuki (2016) Dysregulated YAP1/TAZ and TGF β signaling mediate hepatocarcinogenesis in Mob1a/1b-deficient mice. ***Proc. Natl. Acad. Sci. USA*** 113, E71-E80.
5. Yusuke Nasu, Yoichi Asaoka, Misako Namae, Hiroshi Nishina, Hideaki Yoshimura and Takeaki Ozawa (2016) Genetically Encoded Fluorescent Probe for Imaging Apoptosis in vivo with Spontaneous GFP Complementation. ***Analytical Chemistry*** 88, 838-844.
6. Shunta Nagashima, Junichi Maruyama, Shodai Kawano, Hiroaki Iwasa, Kentaro Nakagawa, Mari Ishigami-Yuasa, Hiroyuki Kagechika, Hiroshi Nishina and Yutaka Hata (2016) Validation of chemical compound library screening for transcriptional co-activator with PDZ-binding motif inhibitors using GFP-fused transcriptional co-activator with PDZ-binding motif. ***Cancer Science*** 107, 791-802.
7. Koichi Fujisawa, Shuji Terai, Taro Takami, Naoki Yamamoto, Takahiro Yamasaki, Toshihiko Matsumoto, Kazuhito Yamaguchi, Yuji Owada, Hiroshi Nishina, Takafumi Noma and Isao Sakaida (2016) Modulation of anti-cancer drug sensitivity through the regulation of mitochondrial activity by adenylate kinase 4. ***J. Exp. Clin. Cancer Res.*** 16, 35 (1), 48.

Department of Stem Cell Biology

Professor	Emi K. Nishimura, M.D., Ph. D.
Associate Professor	Daisuke Nanba, Ph D.
Assistant Professor	Hiroyuki Matsumura, Ph. D.
	Yasuaki Mohri, Ph. D.
JSPS Postdoctoral Fellow (SPD)	Hironobu Morinaga, Ph. D.
Project Assistant Professor	Makoto Fukuda.,Ph. D.

Stem cell systems play fundamental roles in tissue turnover and homeostasis. Our goal is to understand the mechanisms of tissue homeostasis driven by stem cell systems and to apply the knowledge to better understand the mechanisms underlying specific tissue decline by aging, cancer development and other diseases associated with aging. We further aim to apply this knowledge to drug discovery and regenerative medicine using somatic stem cells and the prevention and treatment of age-associated diseases.

1) Identification of stem cells in the skin

The skin is the largest organ in the body. Hair follicles in the skin constantly renew themselves by alternate phases of growth, regression and rest. During this process, mature melanocytes (pigment cells) in hair follicles are replaced by a new cell population every hair cycle. We previously identified the source of those melanocytes, "melanocyte stem cells" (McSC), which are located in the hair follicle bulge and supply mature melanocytes required for hair and skin pigmentation (Nishimura EK et al. Nature 2002). We currently identified McSCs in eccrine sweat glands in non-hair-bearing skin areas as well (Okamoto N et al.PCMR, 2014). Also we are currently searching for the prospective method for identification of epidermal keratinocyte stem cells in mouse and human skin.

2) Mechanisms of stem cell maintenance

The underlying mechanisms of stem cell maintenance is a fundamental issue in stem cell biology and medicine. We previously demonstrated that the niche microenvironment plays dominant role in melanocyte stem cell fate determination (Nishimura EK et al. 2002). We then revealed that hair follicle stem cells (HFSC), which surround McSCs in the hair follicle bulge-subbulge area, serve as a functional niche for McSC maintenance through transforming growth factor β (TGF-β) (Nishimura EK et al. Cell Stem Cell, 2010) (Tanimura S et al. Cell Stem Cell 2011). As intrinsic defects in stem cells such as caused by *Mitf* or *Bcl2* deficiency also induces McSC depletion which leads to the progressive expression of hair graying phenotype, incomplete maintenance of McSCs either by defective signaling from the stem cell niche or by intrinsic defects in stem cells induces the progressive hair graying phenotype.

3) Mechanisms for stem cell aging and tissue aging

Physiological hair graying is the most obvious outward sign of aging in mammals, yet it has been unclear what causes the incomplete maintenance of MsSCs during the course of aging (Nishimura EK et al. Science 2005). We have found that genotoxic stress abrogates renewal of McSCs by triggering their differentiation without inducing stem cell apoptosis nor cellular senescence. Our findings indicated that a "stem cell renewal checkpoint" exists to maintain the quality of the melanocyte stem cell pool (Inomata K, Aoto T et al. Cell 2009). Interestingly, a similar mechanism actually underlies epithelial tissue aging. We recently found that HFSCs also have similar checkpoint mechanism which determines the stemness of HFSCs. We are currently studying the underlying molecular mechanism.

4) Tissue aging driven by stem cell-centric aging program

Hair thinning/loss is a prominent aging phenotype but has an unknown mechanism. We found that hair follicle stem cell (HFSC) aging causes the stepwise miniaturization of hair follicles and eventual hair loss in wild-type mice and in humans. *In vivo* fate analysis of HFSCs revealed that the DNA damage response in HFSCs causes proteolysis of Type XVII Collagen (COL17A1/BP180), a critical molecule for HFSC maintenance, to trigger HFSC aging, characterized by the loss of stemness signatures and by epidermal commitment. Aged HFSCs are cyclically eliminated from the skin through terminal epidermal differentiation, thereby causing hair follicle miniaturization. The aging process can be recapitulated by *Col17a1*-deficiency and prevented by the forced maintenance of COL17A1 in HFSCs, demonstrating that COL17A1 in HFSCs orchestrates the stem cell-centric aging program of the epithelial mini-organ (Matsumura H et al. Science 2016).

5) Development of skin regeneration technology with human skin stem cells and stem cell-targeted small molecules

Human epidermal keratinocyte stem cells can be cultivated under suitable conditions, and generate a progeny large enough to entirely reconstitute the epidermis of an adult human. This has enabled the autologous transplantation of cultured epidermal sheets onto patients with extensive burns. However, the cultured keratinocytes can regenerate only the epidermis and cannot suppress dermal scarring. To develop novel skin regeneration technology, we have investigated human epidermal keratinocytes and found that human epidermal keratinocyte stem cells can be identified *in situ* by analyzing cell motion during their cultivation (Nanba et al., J. Cell Biol., 2015). The identification of keratinocyte stem cells by image analysis is a valid parameter for quality control of cultured keratinocytes for transplantation, and improves the clinical outcome of cell therapy and the efficiency of cell manufacturing for regenerative medicine. Finally, the treatment of skin ulcer and decubitus is an urgent problem in this aging society. We are currently trying to establish the screening system for small molecules that activate stem cells in the wound edge.

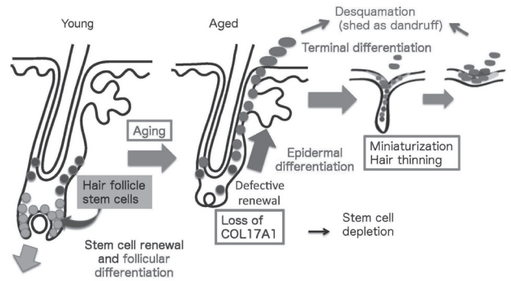

Figure. The mechanisms of hair follicle aging and hair thinning.

Publications

1. Matsumura H, Mohri Y, Binh NT, Morinaga H, Fukuda M, Ito M, Kurata S, Hoeijmakers J, Nishimura EK. Hair follicle aging is driven by transepidermal elimination of stem cells via COL17A1 proteolysis. Science, 351(6273):575-589, 2016
2. Karigane D, Kobayashi H, Morikawa T, Ootomo Y, Sakaki M, Nagamatsu G, Kubota Y, Goda N, Matsumoto M, Nishimura EK, Soga T, Otsu K, Suematsu M, Okamoto S, Suda T, and Takubo K. P38α Activates Purine Metabolism to Initiate Hematopoietic Stem/Progenitor Cell Cycling in Response to Stress. Cell Stem Cell, 19(2):192-204, 2016
3. Hiraoka C, Toki F, Shiraishi K, Sayama K, Nishimura EK, Miura H, Higashiyama S and Nanba D. Two clonal types of human skin fibroblasts with different potentials for proliferation and tissue remodeling ability. J. Dermatol. Sci., 82(2):84-94, 2016

Invited lecture/presentation at international meetings

1. Emi K. Nishimura : New Insights in Stem Cell Research & Age-related Changes in Skin and Hair Pigmentation : 46th Annual ESDR Meeting : (Munich, Germany) September 7-10, 2016
Emi K. Nishimura : Stem cell aging:a clue to understand hair thinning and graying : 1st International Symposium on Stem Cell Aging and Disease : (Tokyo: Ito Hall, The University of Tokyo) June 29, 2016

Department of Immunology

Professor	Takeshi Tsubata, M.D., Ph.D.
Associate Professor	Takahiro Adachi, Ph.D.
Assistant Professors	Naoko Matsubara, Ph.D.
Specially Appointed Junior Associate Professor	Ji-Yang Wang, Ph.D.
Specially Appointed Assistant Professor	Chizuru Akatsu, Ph.D.
Project Researcher	Miao Tang, Ph.D.
	Toshitaro Takata, Ph.D.
	Mohanmmad Aslam, Ph.D.

Normal immune system removes pathogens and cancer cells but does not respond to non-microbial foreign substances or normal self-antigens. Immune responses to non-microbial foreign substances and self-antigens cause allergy and autoimmune diseases, respectively. Immune responses to non-protein antigens play crucial roles in host defense against pathogens such as tuberculosis bacilli and meningococci, and autoimmune diseases such as lupus and immuno-neurological disorders. The mechanisms for immune responses to non-protien antigens are distinct from those to protein antigens, but are largely unknown. Thus, immune responses to non-protein antigens constitute a remaining frontier in immunology research. Followings are our research subjects.

1) Elucidation of the mechanisms for humoral immune responses to glycans, glyco-lipids and nucleic acids-related antigens.
2) Elucidation of the mechanisms for autoantibody production to nucleic acid-related antigens and glycolipids in lupus and immuno-neurological disorders.
3) Elucidation of the role of glycan signals in the regulation of humoral immune responses, and development of synthetic glycosides for immune modulation.
4) Role of cell biological processes such as membrane trafficking and protein degradation in B lymphocyte activation
5) Drug discovery

1. Studies on the mechanisms that regulate production of autoantibodies involved in development of SLE

Autoantibodies to the RNA-related self-antigen Sm/RNP play a crucial role in development of SLE. Previously we developed the method to identify anti-Sm/RNP antibody-producing B cells (Sm/RNP-reactive B cells) by immunohistochemistry and flow cytometry, and demonstrated that Sm/RNP-reactive B cells emerge in the peripheral lymphoid tissues without being selected by tolerance mechanisms at the bone marrow immature B cell stage but negatively selected in the periphery (Kishi et al. PNAS 2012), whereas DNA-reactive B cells are tolerized at bone marrow immature B cell stage. Also we demonstrated that mice deficient in the B cell inhibitory receptor CD72 alone and those deficient in both CD72 and Fas develop moderate and severe SLE-like autoimmune disease, respectively (Xu et al. J. Immunol. 2013). To address how Sm/RNP-reactive B cells escape tolerance during development of SLE, we examined CD72-deficient mice. Sm/RNP-reactive B cells expands in CD72-deficient mice although these mice do not produce anti-Sm/RNP antibodies. In contrast, mice doubly deficient in both CD72 and Fas produced anti-Sm/RNP antibodies. These results suggest that CD72 is involved in self-tolerance of Sm/RNp-reactive B cells, and that anti-Sm/RNP antibody production requires defects in both CD72 and an additional tolerance mechanism such as a defect in Fas. .We further elucidated a novel molecular mechanism by which CD72 prevents development of SLE. CD72 specifically recognizes Sm/RNP, and inhibits activation of B cells that produce anti-Sm/RNP antibodies (Sm/RNP-reactive B cells) thereby preventing development of SLE (Akatsu et al. J. Exp. Med. 2016, see Highlight)

2. Development of CD22-binding sialosides and studies on regulation of B cell activation by CD22 cis-ligands.

CD22 (also known as SIglec-2) is a membrane molecule expressed in B cells, and recognizes sialic acids. CD22 negatively regulates signaling through B cell antigen receptor (BCR) by activating protein tyrosine phosphatase SHP-1. Various membrane-bound lectin molecules including CD22 binds to and are regulated by the glycan ligand expressed on the same cell (cis-ligand). We developed silaosides that specifically binds to CD22 with high affinity. When we inhibited binding of CD22 to cis-ligands using thse sialosides, BCR signaling was down-modulated, suggesting that inhibitory activity of CD22 is negatively regulated by cis-ligands. CD22 has been shown to binds to CD45, which is a protein tyrosin phosphatase expressed in various hematopoietic cells and is heavily glycosilated. Thus, we are currently analyzing whether CD45 regulates CD22 as a cis-ligand by using CD22-deficient B cells.

3. Studies on endocytosis and degradation of BCR

When BCR interacts antigens, BCR is endocytosed together with antigens. BCR endocytosis is considered to down-regulate BCR signaling by removing BCR from cell surface. The endocytosed antigens are degraded in endosome. Resulting antigenic peptides are loaded onto class 2 MHC (MHCII) and are translocated to cell surface leading to T cell recognition of the complex of MHC II and peptides, a process required for T cell help to B cells. Receptor endocytosis has been extensively studied in EGF receptor, and these studies elucidated that endocytosed EGFR forms intraluminal vesicles (IVLs) followed by degradation in lysosome or secretion from the cells as exosomes. When we expressed EGFR in B cells, EGFR is endocytosed upon interaction with EGF, and is rapidly degraded after forming ILVs. In contrast, BCR does not form ILVs after endocytosis, and stays in the endosome for a prolonged time. BCR-localizing endosomes also contain MHCII, suggesting that antigenic peptides are efficiently loaded in these endosomes.

Highlight

Elucidation of a novel mechanism that inhibits production of anti-Sm/RNP antibodies pathogenic in development of SLE

SLE is a prototype of systemic autoimmune diseases and is characterized by production of autoantibodies to various nuclear antigens. Among these autoantibodies, it is already established that antibodies to Sm/RNP, a complex of RNA and nuclear proteins, play a pathogenic role in development of SLE. We demonstrated that the B cell inhibitory receptor CD72 specifically recognizes Sm/RNP and inhibits BCR signaling generated in Sm/RNP-reactive B cells, resulting in inhibition of anti-Sm/RNP antibody production (Figure).

Various immune cells including B cells express various nucleic acid (NAs) sensors that activate cells upon recognition of NAs. NA sensors are involved in induction of anti-micoribal immune responses by recognizing microbial NAs. In contrast, NA sensors also involved in development of SLE through production of autoantibodies to nuclear antigens by recognition of self-NAs. Among NA sensors, TLR7 plays a central role in development of SLE by recognizing Sm/RNP and inducing anti-Sm/RNA antibody production.

NA sensors such as TLR7 and TLR9 are located in endosomes. Self-NAs derived from dead cells are mostly present in a form of free NA and are rapidly degraded by nucleases in body fluid. In contrast, microbial NAs are resistant to nucleases because they are located inside of the microbes, and are therefore exposed to NA sensors after microbes are endocytosed by host cells. Complexes of self-NAs and nuclear proteins such as Sm/RNP are resistant to nucleases, and are recognized by endosomal NA sensors upon endocytosis, leading to immune responses including autoantibody production. Our results demonstrated involvement of the inhibitory receptor CD72 in discrimination of self-NAs complexed with proteins from microbial NAs.

Because CD72 specifically suppresses the autoimmune response of SLE, augmentation of the inhibitory function of CD72 may be an ideal treatment for SLE.

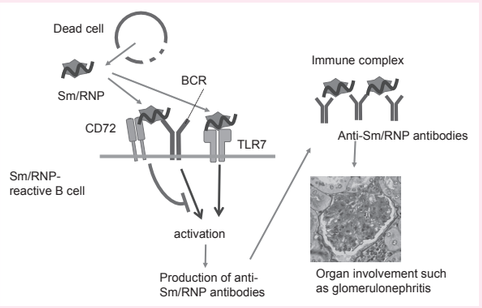

Figure. CD72 mediated suppression of B cell response to Sm/RNP
When Sm/RNP, a complex of nucleic acids and nuclear proteins, is released from dead cells, Sm/RNP is recognized by both TLR7 and B cell antigen receptor (BCR) in B cells that produce anti-Sm/RNP antibodies, leading to activation of Sm/RNP-reactive B cells and production of anti-Sm/RNP antibodies crucial for development of SLE. The inhibitory receptor CD72 binds to Sm/RNP, and inhibits activation of Sm/RNP-reactive B cells thereby preventing development of SLE.

Publications

[original papers]

1. Tsubata, T. (2016): CD22 and CD72 are inhibitory receptors dominantly expressed in B lymphocytes and regulate systemic autoimmune diseases. Z. Rheumatol. 75: 86-89.
2. Li, Y., Takahashi, Y., Fujii, S., Suzuki, A., Tsubata, T., Hase, K. and Wang, J.-Y. (2016): EAF2 mediates germinal center B cell apoptosis to suppress excessive immune responses and prevent autoimmunity. Nat. Commun. 7: 10836.
3. Akatsu, C., Shinagawa, K., Numoto, N., Liu, Z., Konuscan, A.U., Aslam, M., Phoon, S., Adachi, T., Furukawa, K., Ito, N. and Tsubata. T. (2016): CD72 negatively regulates B lymphocyte responses to the lupus-related endogenous Toll-like receptor 7 ligand Sm/RNP. J. Exp. Med. 213: 2691-2706.
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6. Yoshikawa S, Usami T, Kikuta J, Ishii M, Sasano T, Sugiyama K, Furukawa T, Nakasho E, Takayanagi H, Tedder TF, Karasuyama H, Miyawaki A, Adachi T. (2016): Intravital imaging of Ca(2+) signals in lymphocytes of Ca(2+) biosensor transgenic mice: indication of autoimmune diseases before the pathological onset. Sci Rep.6:18738. doi: 10.1038/srep18738.

Department of Molecular Pathogenesis

Professor	Akinori Kimura, M.D., Ph.D.
Associate Professor	Takeharu Hayashi, M.D., Ph.D.
Assistant Professor	Jianbo An, PhD
Research Associate	Taeko Naruse, Ph.D.

Genetic factors, i.e. functional diversity of human genome mainly due to mutations or polymorphisms, are involved in the etiology and pathogenesis of human diseases. Contribution of gene mutations in the disease development is quite large in monogenic diseases showing simple Mendelian inheritance, while multiple gene mutations along with environmental factors are involved in the pathogenesis of multi-factorial diseases. Our goal is to decipher the disease-related genes for intractable or incurable diseases for which etiology and/or pathogenesis are not fully understood and, in addition, to develop novel therapeutic or preventive strategies for the intractable diseases based on the knowledge of disease-related genes.

1. Molecular mechanisms for hereditary cardiomyopathy

We have developed a screening system of mutations in the known 67 genes for hereditary cardiomyopathy. Among the sporadic children cases of hypertrophic cardiomyopathy, a considerable portion could be explained by *de novo* mutations in the sarcomere genes.

2. Molecular mechanisms for atherosclerosis

We generated transgenic mouse lines expressing coronary atherosclerosis-associated MKL1 under the CD68 promoter and found that these mice exhibited abnormality in function of macrophages. In addition, an international collaboration study has confirmed that Lp-PLA2 is not involved in the cardiovascular disease.

3. Molecular mechanisms for arrhythmia

In a collaboration study, IRX3 mutation was identified as a novel disease gene for hereditary arrhythmia.

4. Analysis of MHC genes in human and animals

We have analyzed MHC class I diversities in macaque model for SIV vaccination in detail. In addition, divergence and diversity of MHC class I gene in Penguins were investigated. A phylogenic study suggested an evolutionary feature of Humboldt Penguins.

5. Genome diversity in association with HIV/AIDS

We have investigated natural selection on immune-related genes in the primate evolution. This year, we revealed that APOBEC3H polymorphisms were associated with the susceptibility to HIV-1 infection in Asian Indians.

Highlight

We have investigated divergence and diversity of MHC class I gene in Humboldt Penguins. It is revealed that there are two lineages in birds; one is characterized by multiplication of MHC class I genes, and the other by single MHC class I gene. Humboldt Penguins belong to the latter lineage and the multiplication of MHC class I in the former lineage was suggested to be independently occurred in each species. A phylogenic analysis of MHC class I gene in Humboldt Penguins suggested that there were eight ancestral lineages of MHC class I alleles.

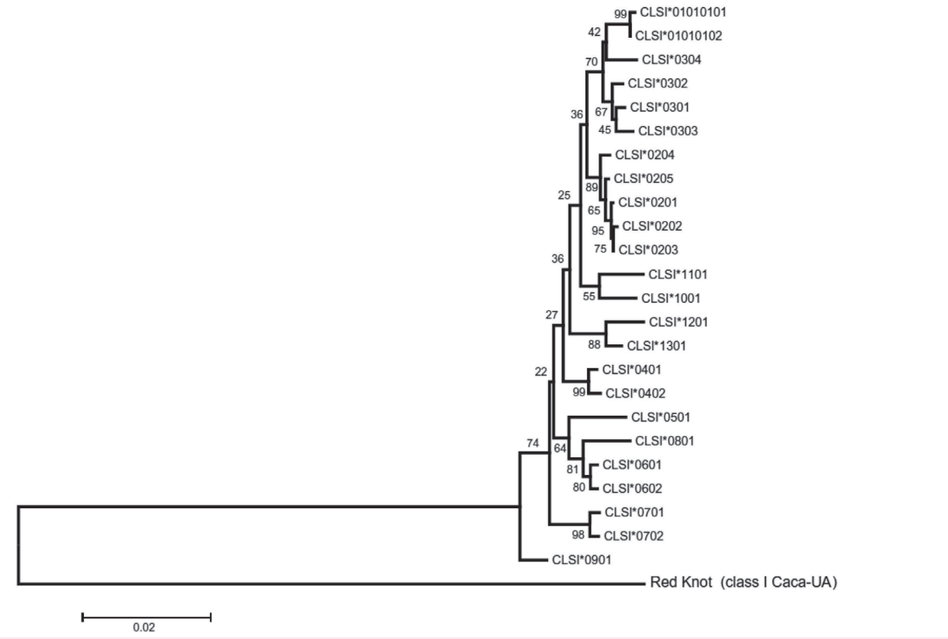

Figure Phylogenic tree of MHC class I genes in birds
The phylogenic study suggested that Penguins were closely related with Red Knot and there were eight ancestral lineages of MHC class I alleles in Humboldt Penguins.

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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.

【Molecular Cytogenetics】

The principal aim of our research is to understand the molecular basis underlying cancer and genetic diseases including multiple congenital anomalies/intellectual Disabilities (MCA/ID). We have contributed as follows;

1. Identification of novel genes including microRNAs responsible for cancer and the clinical development of miRNA-targeting therapeutics in cancer.
2. Understanding the pathogenesis of intractable cancers and genetic disorders based on the integrative omics including systems biology.
3. Establishment of diagnostic devices for the implementation of precision medicine in cancer and genetic disorders.

【Molecular Genetics】

BRCA1/2, products of hereditary breast cancer genes, are associated with genome stability. We analyzed functions of BRCAs and related proteins to reveal the mechanism of breast carcinogenesis.

1. We identified novel functions of BRCA2 in centrosome regulation and cytokinesis.
2. We aimed to establish novel synthetic lethal interactions between BRCA2 and chemical compounds provided by Chemical Biology Screening Center at TMDU.
3. We are investigating the regulation mechanism of BRCA2 expression by estrogen (E2) and estrogen receptor (ER).
4. We have found that BRCA2 binds to histone H3 at the S phase of the cell cycle and regulates methyltransferase activity. and we are analyzing this new function.

【Molecular Epidemiology】

1. We identified that a non-synonymous polymorphism in the transporter associated with antigen processing (TAP2), R651C associates with pulmonary tuberculosis risk.
2. Employing phenome-scan analyses, we found that a polymorphism in the ZFHX3 gene associates with atrial fibrillation, cerebral infarction, and lung thromboembolism.

【Genomic Pathology】

1. We have developed a publicly available software for the global profiling of cancer-stromal interactions by massively-parallel sequencing of cancer xenograft transcriptome. We also examining patient-derived xenograft (PDX), where clinical cancer tissue is directly transplanted into immune-compromised mouse.
2. We are analyzing cancer immuno-genomics of tumor infiltrating lymphocytes to discover biomarkers of cancer immunotherapy.
3. We are investigating relationships between genetic abnormalities in cancer cells and their cellular and architectural abnormalities from digital pathological image and the corresponding genomic information using machine learning technology.

【Epigenetics】

1. We reported the existence of sushi-ichi-related retrotransposon homologue family of genes (*SIRH* family genes) and demonstrated that *Peg10*, *Peg11/Rtl1*, *Sirh7* and *Sirh11*, play essential eutherian-specific functions, such as placental and brain cognitive functions.
2. We have recently reported that most of *SIRH* and *PNMA* family genes are eutherian-specific, thus, suggesting that these newly acquired genes deeply contributed to diversification and establishment of eutherian mammals, including humans.
3. Assisted reproductive technologies, such as *in vitro* fertilization and intracytoplasmic sperm injection, become very popular, however, their effects on human development remain unclear. We have extensively examined pre- and postnatal epigenetic effects caused by such technologies.

【Medical Science Mathematics】

1. As one of striking progresses of big medical-omic data analysis, we exhaustively analyzed whole genomes of 300 hepatocellular carcinoma, and identified six sub-classifications, which revealed significantly different outcomes. Particularly, we found a cluster, with very low recurrence, that has mutations in a novel cancer-related gene.
2. The genetic cause of approximately 20% of LQTS patients remains elusive. With LQTS cases, we performed whole-exome sequencing (WES) and protein-protein interaction network analyses. They revealed new pathogen candidates, half of which directly interact with calmodulin.
3. We compared and characterized four latest commercial WES kits, improved coverage of WES combining the haloplex method, and identified novel pathogenic mutations for congenital neurological diseases and hearing loss.

Department of Molecular Cytogenetics

Professor	Johji Inazawa M.D., Ph.D.
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The principal aim of the Department of Molecular Cytogenetics is to understand the molecular mechanism underlying cancer and genetic diseases including multiple congenital anomalies and/or intellectual disability (MCA/ID). Our research interests are as follows: (1) Identification of genes responsible for cancer and unknown genetic diseases, (2) Development of innovative techniques for the detection of genomic and epigenomic aberrations underlying the pathogenesis of cancer and genomic disorders, and (3) Establishment of practically useful modalities for diagnosis and therapy in personalized medicine in cancer and other intractable diseases. Our goal is to bridge the gap between basic and clinical researches for precision medicine.

I. Precision cancer medicine based on omics and functional research

1. Molecular basis for the development of novel strategies to inhibit tumor metastasis

Cancer metastasis is a multistep process, including genetic and epigenetic events that result in the activation of metastasis-associated genes together with the activation of oncogenes and/or the inactivation of tumor suppressor genes, but the underlying mechanisms of metastasis are still poorly understood. Recently, We have reported that the hypusine cascade promotes cancer progression and metastasis through the regulation of RhoA in squamous cell carcinoma (Muramatsu T et al. Oncogene 2016) and cancer-related microRNAs including miR-1246 which is closely associated with cell invasion by targeting DENND2D in oral squamous cell carcinoma cells (Sakha S et al. Scientific Report 2016).

2. Molecular basis for autophagy-based personalized cancer medicine

It has been suggested that autophagy might contribute to tumor progression through cell survival of cancer cells in microenvironment and resistance to cancer therapy. L-asparaginase (L-asp) has been used for more than three decades and remains as an essential drug in the treatment of patients with acute lymphoblastic leukemia (ALL). Poor response to L-asp is associated with increased risk of therapeutic failure in ALL. However, both the metabolic perturbation and molecular context of L-asp–treated ALL cells has not been fully elucidated. Recently, we identified that treatment with L-asp results in metabolic shutdown via the reduction of both glycolysis and oxidative phosphorylation, accompanied by mitochondrial damage and activation of autophagy. Autophagy is involved in reducing reactive oxygen species (ROS) level by eliminating injured mitochondria. Inhibition of autophagy enhances L-asp-induced cytotoxicity and overcomes the acquired resistance to L-asp in ALL cells. The ROS-p53 positive feedback loop is an essential mechanism of this synergistic cytotoxicity. Our new findings provide the rationale for the future development of combined treatment of L-asp and anti-autophagy drug in patients with ALL (Takahashi H et al. Oncogene 2017).

II. Cancer omics research

Though the "tailor-made Medical treatment Program", we have explored tumor susceptible genes and cancer biomarkers of malignancy for esophageal squamous cell cancer (with TMDU and Aichi Cancer Center), breast cancer (with Cancer Institute Hospital and the Univ. of Tokushima), pulmonary cancer (Nagoya Univ. and Shiga Univ. of Medical Science), colorectal cancer (with CIH and Osaka Univ.), prostate cancer (with Kyoto Univ. and Iwate Medical Univ.) and gastric cancer (with National Cancer Center and UT) in order to establish a personalized cancer medicine.

Furthermore, under the Project for development of innovative research on cancer therapeutics (P-DIRECT), we have performed an integrative analysis of genomics, epigenomics, and gene expression in esophageal squamous cell cancers.

III. Molecular investigation of congenital disorders

Intellectual disability (ID) is a heterogeneous condition affecting 2-3% of the population, often associated with multiple congenital anomalies (MCA). The genetic cause remains largely unexplained for most cases. Since 2005, we have been investigating the causes of MCA/ID of unknown etiology in 645 subjects through the use of chromosomal microarrays. First, we performed a two-stage screening by two in-house bacterial artificial chromosome (BAC) arrays, which identified pathogenic copy number variants (CNVs) in 133 patients. Next, we performed a third screening by SNP arrays in 450 negative cases from the previous screenings, and smaller causative CNVs were detected in 22 subjects. Overall, our three-stage screening allowed the identification of pathogenic CNVs in 155 subjects, which means that 24% of the cases can be explained by alterations in the copy-number state (Uehara et al. J Hum Genet. 2016). Through this project, we also carried out a parallel research following the identification of the CASK gene as a cause of ID and microcephaly with pontine and cerebellar hypoplasia (MICPCH). We recruited 41 additional MICPCH patients and identified CASK aberrations in 32 of them, then clarifying the etiology in 78% of the cases.

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Department of Molecular Genetics

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The hereditary breast cancer responsible genes BRCA 1 and 2 function for DNA double strand break repair. While this function is a guardian who prevents carcinogenesis, it repairs DNA damage caused by anticancer drugs in cancer tissues and reduces cell death induction and results on resistance to treatment. In addition, BRCAs have many other functions to maintain DNA stability, and their dysfunction induces mammary carcinogenesis. Therefore, we will pursue the BRCAs' functions and clarify the mechanism of mammary carcinogenesis, and work on the development of a new method for breast cancer treatment.

1. The physiological role of BRCA2 in centrosome cohesion

The breast cancer suppressor BRCA2 is known to be linked to DNA repair and centrosome duplication. BRCA2 is localized in centrosome through a centrosomal localization signal (CLS). Therefore, we have initiated studies aimed at elucidating the transferring mechanism of CLS, identifying proteins that interact with the CLS. Analysis revealed cytoplasmic dynein 1 heavy chain1 and cytoplasmic dynein 1 light intermediate chain2. As a result, we found that BRCA2 could associate with cytoplasmic dynein1 through an interaction with CLS domain of BRCA2 and transfer to the centrosome.

2. Analysis of estrogen-mediated BRCA2 gene expression mechanism

BRCA2 is expressed in a cell-dependent manner with peak expression in the S phase of the cell cycle. Cell cycle-dependent expression is associated with binding of USF, Elf1 and NF-κB to the *BRCA2* promoter. Recent studies have reported that BRCA2 expression is elevated indirectly in response to the activity of estrogen-estrogen receptor (ER). The role of the estrogen-ER transcription factor in regulation of basal transcription from *BRCA2* promoter was verified in the course of this study. There are eight Sp1 sites and one E-box motif from -492 to +129 bp on the *BRCA2* promoter. The ER-Sp1 complex modulates *BRCA2* transcription under conditions of estrogen stimulation. To determine the potential roles of these Sp1 sites in regulation of *BRCA2* gene transcription, we individually mutated the Sp1 sites on the *BRCA2* promoter and examined estradiol-induced BRCA2 promoter activity in MCF7 cells using the LighSwitch Luciferase Assay.

3. A novel role of BRCA2 in H3K4 histone methyltransferase activity

In this study, we show a novel BRCA2-binding protein on S-phase chromosomes. We observed the chromosomes isolated from S phase cells, followed by immunostaining with anti-BRCA2 antibody. BRCA2 was localized to chromosomes in random-dot pattern. To identify BRCA2-binding proteins on chromosomes, we purified endogenous BRCA2 using anti-BRCA2 antibody from nuclear fractions of MCF7 cells during S phase following sonication treatment and detected the interactome of BRCA2 by RIME (Rapid Immunoprecipitation Mass spectrometry of Endogenous protein), revealing 122 proteins including BRCA2, HP1-γ, mixed-lineage leukemia (MLL) and histone H3. We show that BRCA2 interacts with H3K9me3 in the presence of HP1-γ.

4. FKBP51 regulates cell motility and invasion via RhoA signaling

FK506 binding protein 51 (FKBP51) is involved in multiple signaling pathways, tumorigenesis, and chemoresistance. FKBP51 expression correlates with metastatic potential in melanoma and prostate cancer. We discovered DLC1 and DLC2 as novel interacting partner proteins of FKBP51, using immunoprecipitation and mass spectrometry. These are Rho GTPase-activating proteins that are frequently down-regulated in various cancers. Next, we demonstrated that overexpression of FKBP51 enhances cell motility and invasion of U2OS cells via up-regulation of RhoA activity and enhanced Rho-ROCK signaling. Considered together, our results demonstrate that FKBP51 positively controls cell motility by promoting RhoA and ROCK activation; thus, we have revealed a novel role for FKBP51 in cytoskeletal rearrangement and cell migration and invasion.

Highlight

BRCA2 mediates centrosome cohesion via an interaction with cytoplasmic dynein
(Malik S. *et al.* Cell Cycle 15, 2016)

BRCA2 and cyclin E are localized in centrosome through a centrosomal localization signal (CLS). We uncovered the mechanism by which the centrosomal localization signal (CLS) of BRCA2 interacts with cytoplasmic dynein 1 to localize BRCA2 to the centrosome. In addition, the use of a dominant-negative HA-CLS-DsRed fusion protein, depletion of dynein by siRNA, and the inactivation of dynein by EHNA resulted in the inhibition of the localization of BRCA2 at centrosomes and caused the separation of centrosome pairs during the S phase. Meanwhile, the depletion of both BRCA2 and C-Nap1 caused a larger dispersion of centrosome distances than silencing of C-Nap1 alone. These data highlight that binding between dynein 1 and the CLS of BRCA2 mediates cohesion between centrosomes during S phase and possibly functions as a cell cycle checkpoint (Figure). These data could serve as the foundation for additional research to clarify the role of BRCA2 in cancer development.

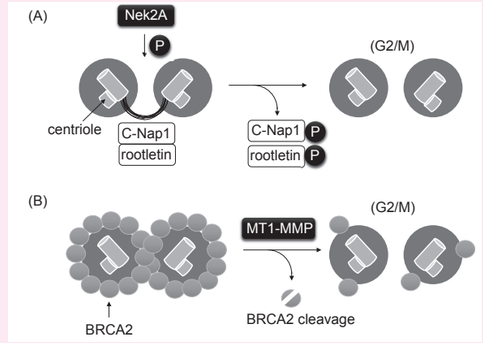

Figure. **Model for BRCA2 localization to the centrosome through its centrosome localization signal.** (A) C-Nap1 and rootletin are required for centriole cohesion during S phase. C-Nap1 and rootletin are substrates of Nek2A and lead to centrosome separation. (B) BRCA2 is required for centrosome cohesion during S phase and cleaved into two fragments by membrane type-1 matrix metalloproteinase (MT1-MMP) during M-phase.

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Department of Molecular Epidemiology

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Many common chronic diseases are multifactorial in that they are caused by multiple genetic and environmental factors. By applying the technology and 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 may contribute to the development of these diseases.

1. Association of polymorphisms of the transporter associated with antigen processing (TAP2) gene with pulmonary tuberculosis in an elderly Japanese population.

The transporter associated with antigen processing 2 (TAP2) gene is involved in the immunological response to tuberculosis (TB) infection. Variations in the TAP2 gene have been associated with TB infection in small population studies in India, Columbia, and Korea. We investigated the association of TAP2 polymorphisms with TB susceptibility in an elderly Japanese population. We analyzed samples from consecutive autopsy cases (n = 1850) registered in the Japanese Geriatric SNP Research database. TB was diagnosed pathologically by TB granuloma on autopsy samples. There were 289 cases and 1529 controls. Twenty-four single nucleotide variations (SNVs), including four missense variations in the TAP2 region, were genotyped using the Illumina Infinium Human Exome BeadChip array. Of the 24 SNVs in the TAP2 gene, rs4148871, rs4148876 (R651C), and rs2857103 showed statistically significant associations with TB susceptibility, and rs4148871 and rs2857103 also showed significant genotypic associations in a dominant allele model adjusted for age, sex, and smoking. Haplotype analysis showed that TAP2 allele *0103 conferred an increased TB risk (OR = 1.48, p = 0.0008), while the TAP2 *0201 allele was protective against TB (OR = 0.73, p = 0.0007). Our results suggest that TAP2 polymorphisms influence TB susceptibility in a Japanese population.

2. Association of ZFHX3 gene variation with atrial fibrillation, cerebral infarction, and lung thromboembolism: An autopsy study.

We aimed to study a single nucleotide polymorphism (SNP), rs2106261, in the transcription factor gene, ZFHX3, in atrial fibrillation (AF) and other related phenotypes by phenome scanning in a Japanese population.

We retrieved consecutive autopsy data (n=2433, mean age=80 years) from the Japanese SNP database for geriatric diseases (JG-SNP). Clinical data, including an AF diagnosis, were collected from medical charts. Genotyping was performed with the DNA chip method. We also analyzed 42 pathological and 26 clinical phenotypes, including cerebral infarctions (CIs) and lung thromboembolisms (LTs), diagnosed by macroscopic inspection during the autopsy.

Among the 2433 patients with available data, 18.6% had AF, 29.4% had CI, and 4.9% had LT phenotypes. The A allele of the rs2106261 SNP was significantly associated with AF, after adjusting for age, sex, diabetes, hypertension, and smoking (AA+AG/GG, OR=1.51, 95%CI: 1.16-1.97, p=0.002). In the entire cohort, CI was not associated with rs2106261 (p=0.14). However, among patients under 80 years old, rs2106261 was significantly associated with CI (AA+AG/GG, OR=1.57, 95%CI: 1.09-2.26, p=0.01). LT was also associated with rs2106261 (AA+AG/GG, OR=1.99, 95%CI: 1.31-3.01, p=0.001). Associations between rs2106261 and CI and LT remained positive after adjusting for the presence of AF, which indicated that this SNP variant might serve as an independent risk marker.

We showed that the ZFHX3 polymorphism, rs2106261 (A allele), was a risk marker for AF and AF-related phenotypes. The roles of this variant in the development of AF and its related phenotypes warrant further investigation.

3. Early gestational maternal low-protein diet diminishes hepatic response to fasting in young adult male mice

Maternal low-protein (MLP) diet in rodents at any gestational period promotes the development of hepatic steatosis in offspring. The MLP-induced steatosis only manifests itself later in life, and it is still unclear whether the young offspring show any signs of past exposure to prenatal adverse conditions. We hypothesized that early nutritional insult would first affect the dynamic responsiveness to nutritional challenges rather than the static state. To address these issues, we analyzed the transcriptome and metabolome profiles of the hepatic response to fasting/refeeding in young male mice offspring to identify changes induced by early gestational MLP diet. Restricted MLP exposure strictly to early gestation was achieved by the embryo transfer method. The fasting-induced upregulation of stress response genes related to protein folding and survival was significantly reduced in MLP pups, suggesting that they are vulnerable to nutritional stress. The transcriptional induction of acyl-CoA thioesterase genes in response to fasting were also diminished in MLP pups. Lipid profiling showed that the hepatic signature of triacylglycerols after a fasting/refeeding cycle was shifted to longer acyl-chains and higher saturation by the MLP diet. Because acyl-CoA thioesterases regulate intracellular ligands for the nuclear receptor peroxisome proliferator activated receptor a (PPAR α the affected transcriptional cascade and the altered lipid profiles may be linked. Taken together, early gestational MLP diet affected the hepatic dynamic response to nutritional stress in seemingly healthy young offspring, accompanied with indirect deterioration of PPAR α action.

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Department of Genomic Pathology

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Research content

Tumor tissue is a complex system composed of tumor cells and multiple types of stromal cells. It is important for the understanding of developmental mechanisms of the disease to reveal the cell-cell interactions and interferences. Our purpose is to understand the dynamic multicellular interactions in such a complicated biological system by measuring a large amount of data at the genomic level, which leads to the identifications of therapeutic targets and biomarkers.

Research introduction

1. Genomic approach for the cancer-stromal interaction

In the department of genomic pathology, we have developed a new method to analyze a wide range of cancer-stromal interactions in tumor tissues which are composed of various types of cells (tumor-stroma interactome). This kind of analysis has been technically difficult to be performed comprehensively and quantitatively. By obtaining the transcriptome data of tumor tissues from tumor bearing mouse, we create gene expression profiles of tumor cells (human cells) and stromal cells (mouse cells) by dividing the sequencing reads into human and mouse. Then, we reveal a global picture of the tumor-stroma interactions by incorporating the protein interaction database (Fig. 1). We are going to identify more integrated interaction profiles using this method.

We are trying to reveal a global picture of interactions between cancer cells and stroma by this method and to identify inevitable signaling pathways on which the tumor microenvironments rely. We identified a number of important signals from stroma to cancer cells by using this method in pancreatic cancer xenograft mouse model.

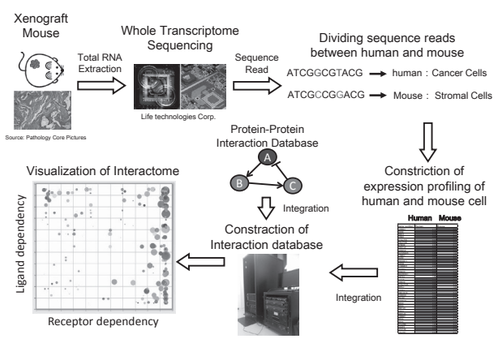

Fig 1. Genomic approach for cancer - stromal interaction

2. Genomics Analysis for Clinical Disease Tissues

In the department of genomic pathology, we have been investigating various clinical disease samples by genomics approaches. By utilizing massively-parallel sequencing, we are obtaining comprehensive data of transcriptome and whole exome sequencing of clinical tissue samples and trying to elucidate the pathogenic mechanism of the diseases defined by genomics aspects.

3. Cancer Immunogenomics

Tumor infiltrating lymphocytes (TILs) seem to play important roles in cancer immunity, as suggested by the finding that the amount of TILs correlates with prognosis in various cancer. However, their functions have remained largely unknown.

In the department of genomic pathology, we try to uncover the functions of TILs in cancer environment by analyzing their antigen receptor sequences using massively-parallel sequencing technology (Fig. 2).

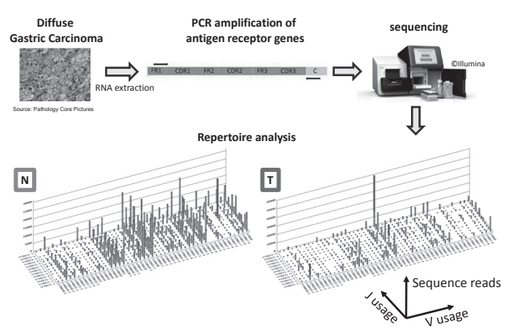

Fig 2. Antigen Receptor Analysis of Tumor infiltrating lymphocytes

4. Functional Genomics Screening

In the Department of Genomic Pathology, we are conducting various kinds of functional genomics screening by combining whole genomic shRNA lentivirus libraries and next-generation sequencing technologies. Our goal is to identify novel therapeutic molecular targets against cancers, and to this end we are exploring possible candidate genes by developing a couple of shRNA screening methods. An example of our screening strategies is a tumor implantation model in which various human cancer cell lines infected with whole-genomic shRNA lentivirus library are inoculated into mice (Fig. 3). In this model, we can quantitatively characterize the populations of cancer cell clones with each shRNA before and after the tumor implantations. We are now identifying candidate genes which significantly suppress cancer cell growths in vivo. We performed numbers of functional genomics screenings targeting various human cancers, having identified some candidate therapeutic target genes.

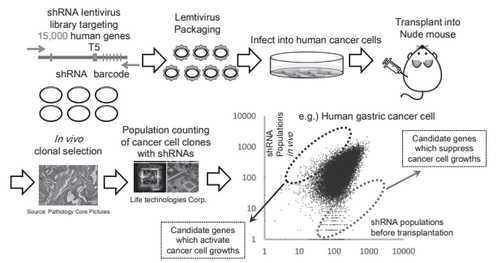

Fig 3. Functional Genomics Screening to Identify Novel Therapeutic Targets against Cancers

5. Image analysis and machine learning in digital pathology

Various genetic abnormalities in cancer cells result in their cellular and architectural abnormalities. Historically, investigating these relationships have provided various insights into cancer biology and gene functions. However, manual investigation of all the relationships in all cancer types by human pathologists is infeasible.

In the department of genomics pathology, we apply deep learning algorithms, which exhibits superior performance in object recognition over conventional machine learning algorithms, to histopathological image analysis of various cancer types. Our aims include inference of clinically relevant somatic mutations from histopathological images, which can be a cost-effective diagnostic tool, and uncovering novel function of genes by investigating the effect of the mutation on the appearance of cancer tissues.

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Original Paper

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Receptor tyrosine kinase amplification is predictive of distant metastasis in patients with oral squamous cell carcinoma.
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Review

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Department of Epigenetics

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Project Lecturer	Lee Jiyoung
Adjunct lecturer	Shin Kobayashi

Introduction: Department of Epigenetics

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

Research subjects

1. Functional differences between paternally- and maternally-derived genomes in mammals (Genomic imprinting etc.)

Imprinted genes, such as paternally and maternally expressed genes (*PEG* and *MEG*) cause functional differences between parental genomes, thus, leading to several genomic imprinting diseases in humans, such as paternal and maternal duplication of human chromosome 14 (Kagami-Ogata syndrome and Temple syndrome, respectively), Silver-Russell syndrome, Beckwith-Widemann syndrome. Much more DNA methylation differences than genomic imprints existed in sperms and oocytes may also play a role in mammalian early embryonic development. We focus on the biological functions of *PEG10* and *PEG11/RTL1* in mammalian development and evolution and on the role of differential gene expressions between paternal genomes in preimplantation development as well as development of therapeutic method of Kagami-Ogata syndrome.

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

Two groups of genes, the *SIRH* (sushi-ichi retrotransposon homologues) and *PNMA* (paraneoplastic Ma antigen) family genes, exist in mammals. *PEG10* is a therian-specific genes, present in marsupials and eutherians but absent in monotremes while *PEG11/RTL1* and all the other genes are eutherian-specific. We demonstrated that *PEG10*, *PEG11/RTL1* and *SIRH7/LDOC1* are essential for establishing mammalian viviparity and *SIRH11/ZCCHC16* is important in cognitive behaviors.

3. Biology of haploid ES cells in mammals

Mouse haploid cell lines provide us new tools for forward/reverse genetics as well as for addressing the relationship between ploidy and cell differentiation. We have already established several haploid ES cells from inbred strains, such as B6 and JF1.

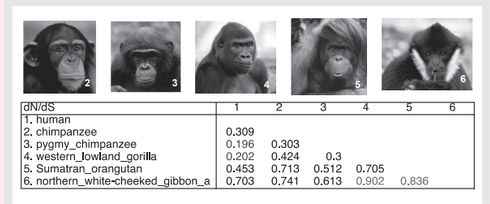
4. New method of analyzing DNA methylation status in genomes

We have developed a new sequencing method, EnIGMA (Enzyme-assisted Identification of Genome Modification Assay), that distinguishes 5-methylcytosine (5mC) and 5-hydroxylmethylcytosines (5hmC) in single DNA fragments. As 5mC and 5hmC may play different roles in gene regulation, this method will provide us precise epigenetic information in the genome.

Highlight

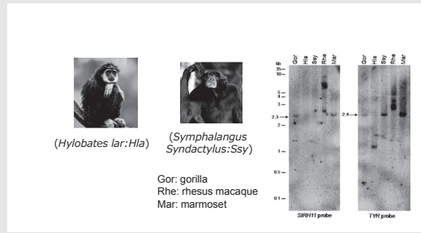
Is an LTR retrotransposon-derived gene, *SIRH11/ZCCHC16*, important for evolution of brain function in eutherians?

Gene targeting of mouse sushi-ichi-related retrotransposon homologue 11/Zinc finger CCHC domain-containing 16 (*Sirh11/Zcchc16*) causes abnormal behaviors related to cognition, including attention, impulsivity and working memory. *SIRH11/ZCCHC16* is highly conserved in three out of the four major groups of the eutherians, euarchontoglires, laurasiatheria and afrotheria, but is heavily mutated in xenarthran species such as sloths and armadillos. Even in the former 3 groups, we found that some lineages have large mutations, lack of the N-terminal half or C-terminal RNA-binding domain, suggesting that this gene has contributed to brain evolution and diversification in eutherians, including humans and mice. For example, in primates, gibbon (Hylobatidae) *SIRH11/ZCCHC16* may not be functional because of accumulation of mutations (Fig. 1, white-cheeked gibbon) or lack of the gene in the genome (Fig. 2, white-handed gibbon and siamang). In New World Mokeys living in South America, all the monkey species lack the N-terminal half, thereby leading to diversification of function estimated from variable dN/dS values.



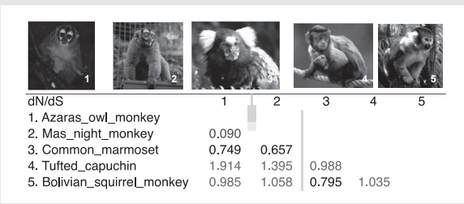
SIRH11/ZCCHC16 may be functionally lost in gibbons (Hylobatidae) among Hominoidea. Loss of the CCHC RNA-binding domain seems to cause pseudogenization.

Figure 1. **Mutation of SIRH11/ZCCHC16 in white-cheeked gibbon (Lack of the C-terminal domain).** Gibbons are a close relative of the hominids, including humans. A nonsense mutation leading to loss of the C-terminal CCHC RNA-binding domain were observed in white-cheeked gibbon. The dN/dS ratio* in the pairwise comparison among the hominoids indicated that *SIRH11/ZCCHC16* is highly constrained among humans, chimpanzees and gorillas (0.20–0.42), while the values of gibbon (*Nle*) compared to humans and chimpanzees are higher (0.61–0.74), and those compared to the gorilla and orangutan are close to 1 (0.84–0.90), suggesting that the truncated *Nle* ORF has been subjected to a lesser degree of purifying selection compared to other hominoid members having the full-length SIRH11/ZCCHC16. * dN/dS<1: conservative, dN/dS~1: neutral or loss of function, dN/dS>1: positive selection



Loss of *SIRH11/ZCCHC16* gene in two other gibbon species, white handed-gibbon and siamang

Figure 2. **Lack of SIRH11/ZCCHC16 gene in two other gibbon species.** Southern blot analysis of two gibbon species, white-handed gibbon and siamang, confirmed that there was no corresponding band to *SIRH11/ZCCHC16*, suggesting that a large deletion or profound structural change had occurred in these two gibbon species. All these results (Figs 1 and 2) demonstrate that gibbons in at least three out of four genera do not possess the normal full-length SIRH11/ZCCHC16 ORF as a result of deletion/structural changes or the lack of a CCHC RNA-binding domain, supporting the notion that the gibbon *SIRH11/ZCCHC16* gene is not functional and instead has become a pseudogene.



SIRH11/ZCCHC16 is functionally diverged among Platyrrhini (New World monkeys) After loss of N-terminal half, their truncated SIRH11/ZCCHC16s seem functionally changed and diverged in a species-specific manner.

Figure 3. **Mutation of SIRH11/ZCCHC16 in New World Monkeys (Lack of the N-terminal half).** The pairwise dN/dS analysis among five New World monkey species demonstrated that the truncated SIRH11/ZCCHC16 ORFs are highly constrained (dN/dS = 0.09) between the two night monkeys (the Azaras owl monkey and Ma's night monkey). However, those of the tufted capuchin are variable: greater than 1 (1.9 and 1.4) to the night monkeys, close to or less than 1 (0.99 and 0.80) to the common marmoset and Bolivian squirrel monkey. Those of the Bolivian squirrel monkey are consistently close to 1 (0.80–1.1), suggesting neutral evolution. Thus, it is possible that functions of the truncated ORFs in Platyrrhini were diversified to a great extent, presumably because of species-specific adaptation after the structural change of the N-terminus deletion in a common Platyrrhini ancestor.

Publications (Original papers)

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Department of Medical Science Mathematics

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Research Summary

Recently, medical application of rapidly progressing omic profiling technologies and, in particular, the promotion of personalized/precision/preventive medicine have been keenly desired. Traditional therapies do not consider the individuality of each patient sufficiently. Our department overcomes such medical science issues by using a combination of mathematics and computational sciences. Nowadays, biomedical big data of clinical and omic profiles are collected from hospitals and medical institutions. First, applying data-mining methodologies, we explore etiologies of intractable diseases, e.g. cancer, common diseases, and neurodegenerative diseases. Next, we classify each disease into finer categories through molecular profiles, and understand disease causing mechanisms through a systems approach. In this way, we can collect knowledge of disease incidence and progression based on clinical and omic data. Last, we apply mathematical methods, e.g, machine learning techniques, to predict optimized therapy for each patient when she/he visits a hospital/medical institute, and we can also apply these methods to disease prevention based on an individual's health check records.

Research Projects

1. Exploring etiologies, sub-classification, and risk prediction of diseases based on big-data analysis of clinical and whole omics data in medicine (CREST, JST)

Toward the goal of personalized/precision medicine, we fully integrate omics, clinical, and molecular datasets into big data. We develop methodologies and utilize advanced statistics and computer science techniques for medical big data analysis. Our plan: (a) standardization, integration, and management of big data, (b) exploration of the compound factors of disease, (c) sub-classification of disease, and (d) prediction of disease and the optimum therapy for individuals. Data resources include: (a) BioBank data, which includes genomic and clinical data, (b) multi-omics and clinical data of cancer patients, (c) whole genome/exome sequences and clinical data of liver cancer patients, (d) prospective genome cohort, with abundant drug application data, of liver disease patients, (e) prospective genome cohort, with precise follow-ups after drug application, of rheumatoid arthritis patients, and (f) molecular DBs. Our department proposes new methodologies towards these goals. In addition to several successful results from the analysis of liver cancer, we recently observed a significant correlation between the molecular clustering of omic data and clinical information,

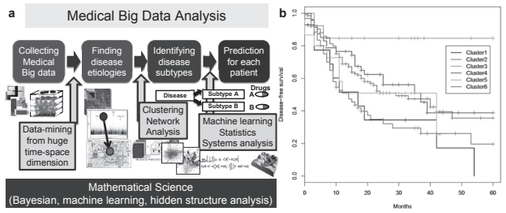

Figure. **Medical Big Data Analysis for Precision Medicine.**
(a) Common analysis steps and methodologies. (b) Application to omics data from 300 hepatocellular carcinoma cases revealed six clusters, which showed significantly different disease-free survival rates (***Nature Genetics***, 48, 500-509 (2016)).

e.g. survival time, from the liver cancer omic analysis [1].

2. Genome sequencing analyses of heart disease reveal candidate pathogenic mutations in new genes

Mutations in the coding sequence of *SCN5A*, which encodes the cardiac Na^+ channel α subunit, have been previously associated with inherited susceptibility to various arrhythmias. However, it is unknown whether variants in the promoter and regulatory regions of *SCN5A* modulate the risk of arrhythmias. We resequenced the core promoter region and the regulatory regions of *SCN5A* transcription in 1298 patients with arrhythmia phenotypes, and identified 26 novel rare variants in the *SCN5A* promoter from the 29 patients. Using a luciferase reporter assay, we functionally demonstrated that 6 of the 26 variants are associated with decrease of the promoter activity. We also identified rare variants in the regulatory region that were associated with atrial fibrillation, and the variant decreased promoter activity [12].

Mutations in *ANK2* have been reported to cause various arrhythmia phenotypes. However, only a small number of ANK2 mutation carriers, mainly in Caucasians, have been reported with limited clinical features, and to our best knowledge no Japanese patients with ANK2 mutations have been reported. We analyzed 535 probands with inherited primary arrhythmia syndrome for ANK2 using NGS. Of the 553 probands, 12 probands were found to carry 7 different heterozygous *ANK2* mutations. Surprisingly, 4 of the 8 LQTS patients had the acquired type of LQTS (aLQTS), and total of 7 of the 12 patients had documented malignant ventricular tachyarrhythmias. We showed that various ANK2 mutations are associated with a wide range of phenotypes, representing "ankyrin-B" syndrome in Japanese [11].

3. Investigation of pathogenic mutations for congenital neurological diseases and hearing loss.

We recently established a consortium with the aim of identifying disease-causing mutations of congenital neurological diseases and hearing loss. To that end, we performed whole-exome sequencing (WES) analysis on ~160 families (~500 individuals). We identified some novel pathogenic mutations for the diseases [5-10].

Publications

[Original articles]

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Frontier Research Unit
Laboratory of Gene Expression

Associate Professor Hidehito KUROYANAGI
Project Assistant Professor Sharmin HASAN (-July, 2016),
Shotaro WANI (April, 2016-)

Recent whole genome sequence analyses revealed that a high degree of proteomic complexity is achieved with a limited number of genes. This surprising finding underscores the importance of alternative pre-mRNA splicing, through which a single gene can generate structurally and functionally distinct protein isoforms. Based on recent transcriptome analysis, >90% of human multi-exon genes produce multiple mRNA isoforms. Regulation of the splice site choice through so called "splicing codes" provide a versatile mechanism for controlling gene expression and for generation of the proteome diversity. We are trying to decipher the splicing codes in living organisms.

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

We have developed a transgenic fluorescence alternative splicing reporter system that enables visualization of alternative pre-mRNA processing patterns in vivo (Nat Meth, 2006; Nat Protoc, 2010). With this reporter system, we have visualized spatiotemporal profiles of tissue-specific and/or developmentally regulated alternative splicing events in living nematode worms *C. elegans*. By isolating and analyzing mutant worms defective in the color profiles, we have identified *trans*-acting factors and *cis*-elements involved in the splicing regulation (Mol Cell Biol, 2007; Genes Dev, 2008; PLoS Genet, 2012; PLoS Genet, 2013; NAR, 2016; Nat Commun, 2016) (see figure). We have solved solution structure of two RNA-binding proteins cooperatively recognizing their target RNA stretch by sandwiching a hydrophobic guanine base (Nat Struct Mol Biol, 2014). Through these studies, we now realize that molecular mechanisms of the alternative splicing regulation are conserved throughout metazoan evolution.

Global Search for Target Events of Tissue-Specific Splicing Factors.

We are searching for alternative splicing events that are affected in the splicing factor mutants through transcriptome analyses by utilizing a next generation sequencer. We found new target events for a neuron-specific splicing factor UNC-75 and identified its cis-elements through bioinformatic and reporter analyses (Nucleic Acids Res, 2013). We have also identified natural non-coding mRNA isoforms that are rapidly degraded by nonsense-mediated mRNA decay (NMD) *in vivo* (NAR, 2016).

Heart-Specific Alternative Splicing of the *TTN* Gene and Dilated Cardiomyopathy.

Dilated cardiomyopathy (DCM) is caused by mutations in sarcomere protein genes including *TTN*. Titin, encoded by the *TTN* gene, is a huge sarcomeric protein; passive tension of myofibers is mainly attributed to the titin protein. The *TTN* gene consists of 363 exons and its pre-mRNA splicing patterns and apparent molecular weight of the titin proteins are developmentally regulated and vary between cardiac muscles and skeletal muscles. In DCM models, the ratio of the titin mRNA and proteinisoforms are affected, suggesting correlation between the titin isoform change and DCM pathology. We constructed a fluorescence reporter minigene to successfully visualize the heart-specific splicing regulation of the *TTN* gene. By utilizing this reporter, we found that some point mutations in an RNA-binding protein RBM20 disrupt its function as a splicing factor for the heart-type *TTN* pre-mRNA splicing.

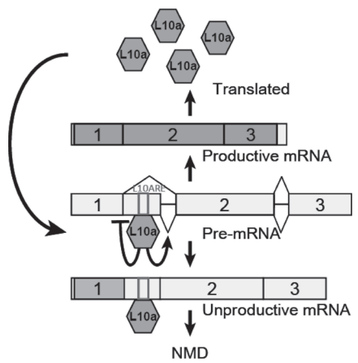


Figure. Ribosomal protein L10a negatively regulates expression of its own mRNAs as a splicing regulatory factor.

Publications

Original Articles

1. Takei S, Togo-Ohno M, Suzuki Y, Kuroyanagi H. Evolutionarily conserved autoregulation of alternative pre-mRNA splicing by ribosomal protein L10a. Nucleic Acids Research. 44: 5585-5596, 2016.
2. Tomioka M, Naito Y, Kuroyanagi H, Iino Y. Splicing factors control *C. elegans* behavioural learning in a single neuron by producing DAF-2c receptor. Nat Commun. 7: 11645, 2016.

Project Research Unit

Associate Professor Michinori Kubota

Effects of salicylate on neural activities to repetitive sounds with different repetition rates were investigated in the primary auditory cortex (AI) of the guinea pig using optical imaging with a voltage-sensitive dye (RH795). Salicylate, the active ingredient of aspirin, can cause a sensory hearing loss by reducing outer hair cell electromotility.

Activity patterns to repetitive sounds with different repetition rates (8 kHz tone of 25 ms duration or click, 4-20 Hz repetition rate, 75 dB SPL) were recorded from the AI on both sides before (control) and 8 hours after the intraperitoneal injection of 200 mg/kg salicylate. In control condition, the repetition-rate transfer functions (RRTFs) of the 2nd, 3rd and 4th peaks in response to tones had band-pass characteristics with a peak at 6 Hz while RRTFs in response to clicks had band-pass characteristics with a peak at 8 or 10 Hz showing a sharp drop-off. At 8 hours after the salicylate injection, the RRTFs of the 4th peak in response to tones frequently showed the large peak at 6-8 Hz and the small peak at 16 Hz in the right AI. These results suggest that activities to repetitive sounds could be affected by outer hair cell electromotility.

Publications

Hosokawa Y, Kubota M, Sugimoto S, Horikawa J. Salicylate-induced changes of the neural activity to repetitive sounds in the primary auditory cortex of guinea pigs observed by optical recording. J Physiol Sci, Vol. 66, Suppl. 1, S174 (2016).

Laboratory for Integrated Research Projects on Intractable Diseases

Advanced Technology Laboratories

Laboratory for Integrated Research Projects on Intractable Diseases

IBD project, Laboratory for Integrated Research Projects on Intractable Diseases

Professor Shigeomi SHIMIZU
Akinori KIMURA
Toshiaki OHTEKI
Assistant Professor Yusuke NAKANISHI

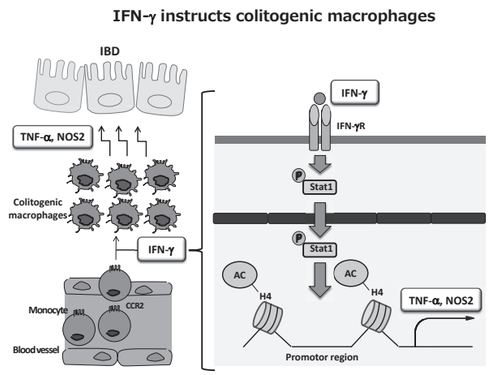
Summary

Inflammatory bowel disease (IBD) primarily includes ulcerative colitis and Crohn's disease. Both usually involve chronic inflammation, severe diarrhea with bloody stool and pain. Our goal is to understand the mechanism of IBD development and find the new therapies and treatments of the disease.

Research Project

Macrophages are an essential component of the innate immune system. Recent discoveries revealed that macrophage functions are not restricted to the host defense and clearance of apoptotic cells, rather extended to tissue development, homeostasis and repair. TNF-α and iNOS are representative inflammatory mediators that contribute to the development of colitis, suggesting that a blockade of these mediators may be therapeutic. We previously reported that $CD11b^{+}CD11c^{int/-}$ cells infiltrated into the inflamed colon contain macrophages, monocytes, eosinophils and neutrophils at the peak of inflammation. Of these, only monocytes and macrophages produced the colitogenic mediators TNF-α and iNOS (*Mucosal Immunol.,* 2016). However, how monocytes and/or macrophages gain the colitogenic property during the development of colitis is totally unknown.

Using a chemically induced colitis model, we showed that $Ly6C^{+}$ $MHC^{-}II^{+}$ macrophages strongly expressed representative these colitogenic mediators. We found that IFN-γ–Stat1 pathway was required for generating colitogenic macrophages, given that *Stat1* deficient mice showed less severe colitis with fewer colitogenic macrophages. Notably, IFN-γ induced histone acetylation at the promoter regions of the *Tnf* and *Nos2* loci in macrophages, indicating that IFN-γ–dependent epigenetic regulation instructs the development of colitogenic macrophages *in vivo*. Collectively, our results provide the essential mechanism by which dysregulated macrophages develop at the colon mucosa during inflammation, and suggest a new drug target for treating IBD.



Publications

Nakanishi Y, Sato T, and Ohteki T. Commensal Gram-positive bacteria initiates colitis by inducing monocyte/macrophage mobilization *Mucosal Immunology* 2015.152-60.

Research Project on Precision Medicine for Head and Neck and Esophageal Squamous Cell Carcinoma.

Professor Johji Inazawa

Lymph node metastasis (LNM) of esophageal squamous cell carcinoma (ESCC) is well-known to be an early event associated with poor prognosis in patients with ESCC. Recently, tumor-specific aberrant DNA methylation of CpG islands around the promoter regions of tumor-related genes has been investigated as a possible biomarker for use in early diagnosis and prediction of prognosis. However, there are few DNA methylation markers able to predict the presence of LNM in ESCC. To identify DNA methylation markers associated with LNM of ESCC, we performed a genome-wide screening of DNA methylation status in a discovery cohort of primary ESCC tissues and their paired normal esophageal tissues using the Illumina Infinium HumanMethylation450 BeadChip. In this screening, we focused on differentially methylated regions (DMRs) that were associated with LNM of ESCC, as prime candidates for DNA methylation markers. We confirmed that two makers (Gene-1 and Gene-9) were highly methylated in LNM-positive tumors in an independent set of ESCC cases. These results suggested that Gene-1 and Gene-9 may be useful epigenetic biomarkers for the prediction of the presence of LNM in ESCC.

DOHaD research towards preventive and preemptive approach against chronic intractable disease

Principal researcher Noriko Sato
Molecular Epidemiology Katsuko Sudo, Chihiro Imai
Comprehensive Reproductive Medicine Naoyuki Miyasaka
Periodontology Yu-ichi Izumi, Sayaka Katagiri
Epigenetics Takashi Kohda

Research project

Developmental Origin of Health and Disease (DOHaD) is the concept that the process through which the environment encountered before birth and/or infancy shapes the long-lasting bodily function and physiology. Epidemiological studies have shown that inadequate conditions in early stage of life can predispose us to lifelong diseases, which are developed later. It is crucial to accumulate genomic and epigenomic epidemiological data of prospective birth cohorts, and to analyze how environment interacts with fetal genome and modulates its phenotype, which is still a burgeoning field of research in Japan. Our Birth Cohort – Gene and ENvironment Interaction Study of TMDU (BC-GENIST) will sort out the current environmental conditions possibly threatening mother and child health. We will identify the interindividual epigenetic differences caused by the interaction between the genetic polymorphisms and environmental variables. In order to elucidate the molecular mechanisms how the prenatal conditions form the future disease phenotype, animal models are being investigated. Particularly, we are currently studying the effects of parental ageing on embryo development and the effects of periodontal disease on pregnancy and metabolism.

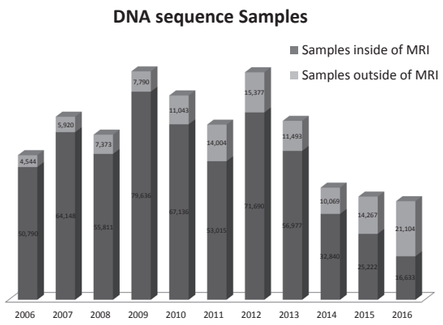
Research findings

1. More than 50 pregnant women agreed to participate in BC-GENIST (by Jan. 2017). The average maternal age and energy intake were 33 ± 3.6 (years old) and 1621 ± 257 (kcal/d), respectively. Their dietary intake was below the recommended requirement (reference:1800) for pregnancy demands. (http://hikumano.hama-med.ac.jp/dspace/handle/10271/3130)
2. The latent trajectory analyses identified four distinct trajectory patterns in the fetal growth rate. The pilot experiment found that the neonatal DNA methylation was associated with fetal growth rate and gestational age after adjusting for cis-SNPs.

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. Followings are the achievements in 2016.



1. Sequencing analyses

A total of 37,737 samples from 2,999 researchers were sequenced in the year of 2016. Among them 21,004 (55.6%) samples were requested by researchers outside the medical Research Institute (see below). Analysis by using next generation sequencing equipment (Ion Torrent PGM) has been started in 2013 and 24 runs were done in the year of 2016. Library preparation service for next generation sequencing has been started in 2015 and 24 samples were done in the year of 2016.

2. Equipment under the management of the Genome Laboratory.

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

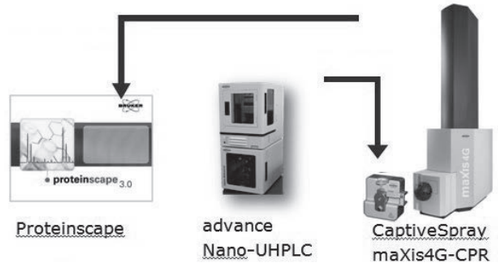
3. Introductory seminars

Introductory seminars were done for use of instruments (3 times).

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 equipments written below.

For proteome analysis, we have a 2D-electrophoretic system, LC-MSMS system and Biacore system in this laboratory. We can accept the consignment analysis of proteins with the two dimension electrophoresis and the mass spectrometry by request of researchers of this university. In addition, we can give technical advices on cytometry and proteome researches to young scientists who wish to start own research.



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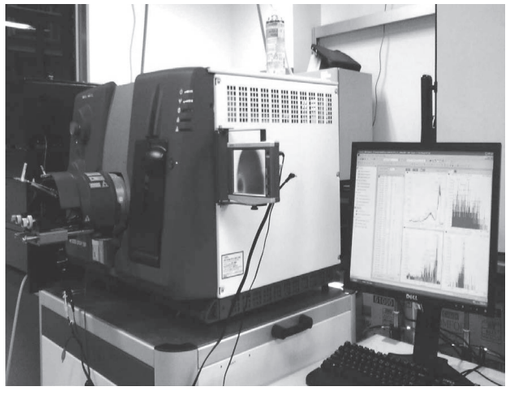
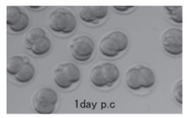


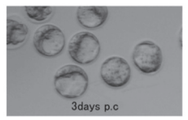
Qtrap5500 ABSCIEX

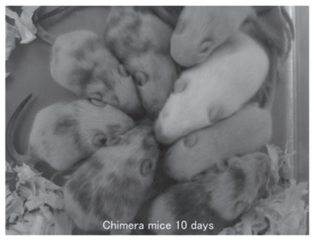
Laboratory of Recombinant Animals

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 the Recombinant Animal Laboratory, excellent technical staffs generate, clean up, maintain and store recombinant mice to facilitate the biomedical research in Medical Research Institute. Since 2016, using genome editing techniques, 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-325E, HM-335E (Microm)
- Vibrating microtome … PRO7 (D.S.K.)
- Automated Tissue Processor
  … RH-12DM (Sakura Finetek)
  Excelsior ES (Thermo Scientific)
- Tissue-embedding-station
  … Histostar (Thermo Scientific)
- Real-time PCR … 7500、7900HT (Applied Biosystems)
- Laser microdissection … LMD7000 (Leica)
- X-ray System … RX-650 (Faxitron)
- Stereo microscope … SZX-16 (Olympus)

<<seminars>>

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

In this fiscal year, seminars were done twice each time.

Laboratory of Bioresource

Bioresource Lab. of Medical Research Institute was established in 2004, to support researchers and help postgraduates in cell culture. The center safely supply domestically or internationally well-recognized cultured cell lines as research materials. All of the cell lines handled are collected after exchanging MTA with original developers. EB-virus transformed cell lines are also established with B-lymphocytes from patients with intractable diseases including genomic disorders after written informed consent from each of the patients or their parents and also with approval of the Internal Review Board on ethical issues.

Laboratory for Structure Analysis

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

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. Now the Laboratory is equipped with basic and state-of-the-art research instruments, including high-speed cell sorters (MoFlo Legacy and MoFlo XDP), confocal laser scanning microscopes (FV10i-W for time-lapse images, and FV10i-DOC for one shot images).

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

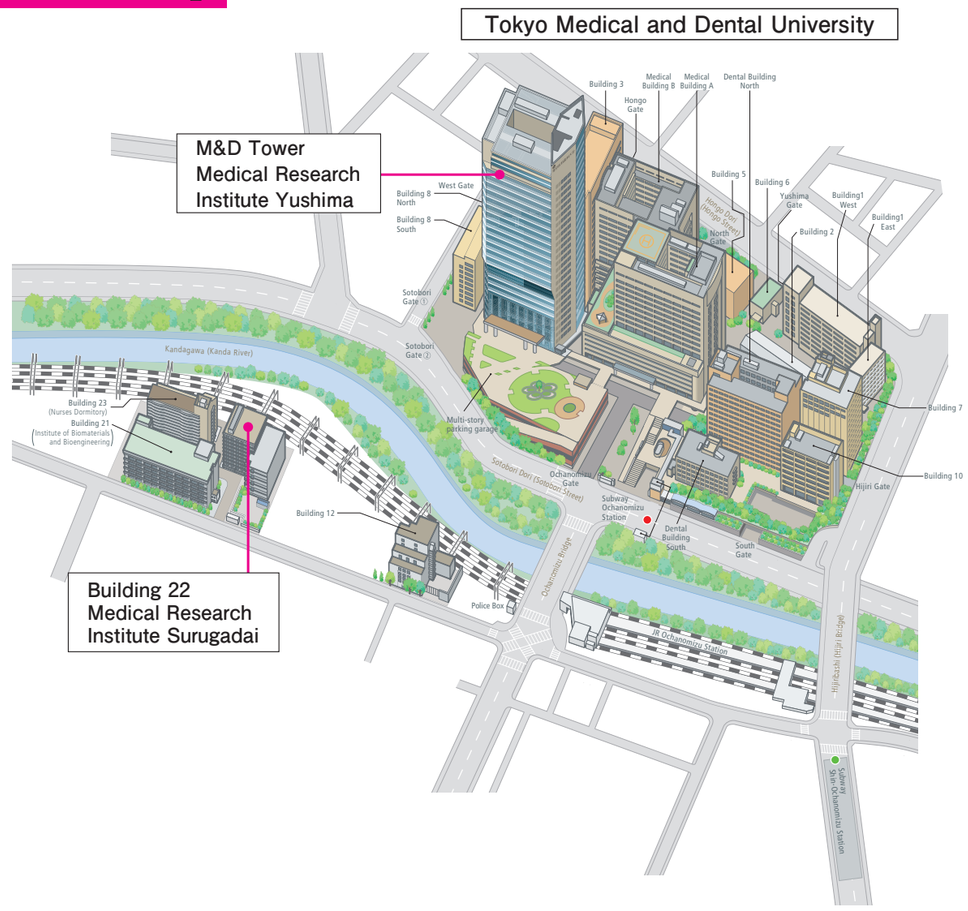
The use of the equipment and cell sorting service are available to researchers in other departments within the University and those outside since August 1st, 2013. The number of users is increasing every year.

The number of overall use cases was 424 in the year of 2016. We held 3 short courses for beginners to help them use the equipment.

Advisory Committee Members

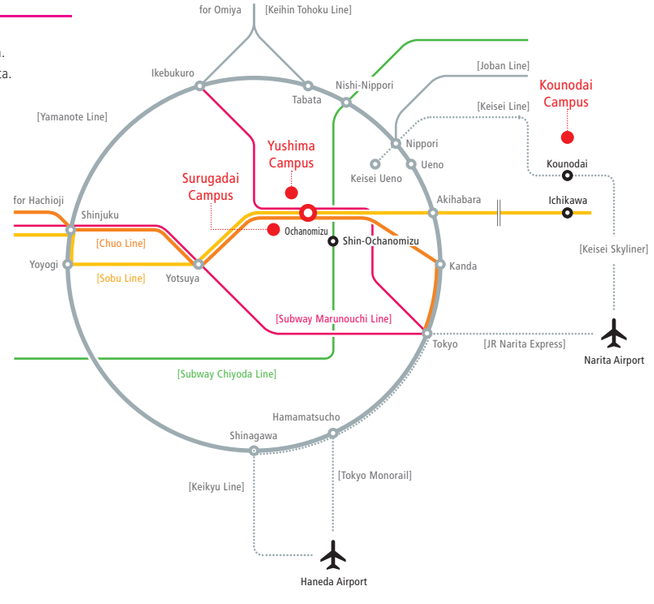
GO Mitiko	Trustee Nagoya University
SASAZUKI Takehiko	University Professor Kyushu University
TANAKA Takaharu	President Hoshi University
TANIGUCHI Masaru	Special Advisor RIKEN Center for Integrative Medical Sciences
NAGAI Ryozo	President Jichi Medical University
NAKAGAMA Hitoshi	Director National Cancer Center Research Institute
NAGANO Tetsuo	Vistiting/Emeritus Professor Drug Discovery Initiative The University of Tokyo
NISHIKAWA Shin-ichi	Advisor JT Biohistory Research Hall

Access Map



Nearest Stations

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



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