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Annual Report

2021

Preface

It is our pleasure to present Annual Report 2021 of the Institute of Medical Science, The University of Tokyo (IMSUT). The predecessor organization of IMSUT was founded in 1892 as the Institute of Infectious Diseases (IID) by Dr. Shibasaburo Kitasato and incorporated into the University of Tokyo in 1916. In that era, infectious diseases were the greatest threat to public health and the IID was the center for research into infectious diseases in Asia and a top center for the world, as well. As its successor, IMSUT has been contributing to basic, translational, and clinical research efforts fighting against SARS-CoV-2 and COVID-19, as you will see in the report.

In 1967, this institute received its present name when we reorganized it to encompass a wider range of medical sciences to meet the demands of modern medical care after World War II. Now, based on a history and tradition of over 130 years since the IID's foundation, IMSUT's mission has grown to contribute to the development and welfare of human society through research in cutting-edge medical science and the implementation of state-of-the-art medical care. To achieve this mission, IMSUT promotes interdisciplinary research and develops it into a wide range of practical applications, from the establishment of artificial intelligence (AI) and supporting the most advanced AI medical care, to the development of drugs, including gene, virus, and vaccine therapies, cell and organ transplantation using stem cells and iPS cells, and new dimensions of genomic medicine.

For over a century, IMSUT has handed down the three guiding principles established by Dr. Shibasaburo Kitasato: "practical studies" which benefit society; diverse and inclusive "comprehensive research;" and "disease prevention." Together, these three principles form the basis of healthcare, and remain the foundation of our rapidly developing current research. IMSUT is pioneering new intellectual horizons by efficiently utilizing an enormous quantity of information with AI. We are accelerating the efficient utilization of this new technology, as we promote the establishment of AI for medical science research with our specialized supercomputer, "SHIROKANE", which has already led to AI-guided medical care for hematopoietic tumors in our in-house hospital.

Importantly, IMSUT was authorized in 2018 as Japan's only International Joint Usage/Research Center serving the life science field by the Minister of Education, Culture, Sports, Science and Technology. Based on its highly appreciated activities and achievements, IMSUT was reapproved this year to continue the center program for the next term of six years. By utilizing this platform, we are supporting 25 international joint research projects in fiscal year 2021. As a world-leading medical science institute, we fervently desire to further contribute to the development of global communities of basic, translational, and clinical research.

This annual report summarizes our scientific achievements in 2021. I sincerely hope that these achievements will inspire yet further advances, promote worldwide collaborations with our scientists, and ultimately contribute to improve health care around the world.

January 2022

Yuji Yamanashi, Ph.D.
Dean
The Institute of Medical Science
The University of Tokyo

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機構および職員

〈as of December, 2021〉

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Department of Cancer Biology　癌・細胞増殖部門

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Advanced Clinical Research Center　先端医療研究センター

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Research Center for Asian Infectious Diseases ····· 215

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Associate Professor	Tkiko Nagamura-Inue, M.D., Ph.D.	准教授	博士(医学)	長　村　登紀子
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Head of Laboratory II	Yasunori Ota, M.D., Ph.D.	Ⅱ室室長	博士(医学) 大 田 泰 徳

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IMSUT-HLC セルプロセッシング施設

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RESEARCH ACTIVITIES

Department of Microbiology and Immunology

Division of Infectious Genetics
感染遺伝学分野

Professor	Kensuke Miyake, M.D., Ph.D.	教　授	医学博士	三　宅　健　介
Associate Professor	Shin-Ichiroh Saitoh, Ph.D.	准教授	博士(医学)	齋　藤　伸一郎
Project Associate Professor	Ryutaro Fukui, Ph.D.	特任准教授	博士(医学)	福　井　竜太郎
Assistant Professor	Takuma Shibata, Ph.D.	助　教	博士(医学)	柴　田　琢　磨
Assistant Professor	Ryota Sato, Ph.D.	助　教	博士(医学)	佐　藤　亮　太

Immune cells express multiple Toll-like receptors (TLRs) that are simultaneously activated by various pathogen products derived from microorganisms and viruses. Recent reports have shown that an imbalance in TLR responses results in autoimmune disease. Nucleic acid-sensing (NA-sensing) TLRs detect not only bacterial and viral NAs, but also host-derived NAs. To avoid excessive immune responses to host-derived NA, there may be regulatory mechanisms that control TLRs expression, localization, and function. From this speculation, it is considered that TLRs are involved not only in autoimmune diseases but also in the onset of many diseases. Our research focuses on regulatory mechanisms that control TLR-mediated recognition of pathogenic ligands and identification of endogenous ligands. Our research goal is to elucidate the pathogenic mechanisms of histiocytosis and autoimmune diseases that are thought to be caused by TLRs.

1. Elucidation of the pathogenic mechanism of overlap syndrome with both autoimmune hepatitis (AIH) and primary biliary cholangitis (PBC) symptoms

Shin-Ichiroh Saitoh [1], Kenichi Harada[3], Yoshiko Mori Saitoh [1], Ge-Hong Sun-Wada [4], Yoh Wada [5], and Kensuke Miyake [1, 2]

[1]Division of Infectious Genetics, Department of Microbiology and Immunology. [2]Laboratory of Innate Immunity, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minatoku, Tokyo 108-8639, Japan.

[3]Department of Human Pathology, Kanazawa University School of Medicine, Kanazawa 920-8640, Japan.

[4]Department of Biochemistry, Faculty of Pharmaceutical Sciences, Doshisha Women's College, Kohdo, Kyotanabe, Kyoto 610-0395, Japan.

[5]Division of Biological Science, Institute of Scientific and Industrial Research, Osaka University, 8-1 Mihogaoka, Ibaraki, Osaka 567-0047, Japan.

Autoimmune hepatitis (AIH) and primary biliary cholangitis (PBC) are intractable diseases whose pathogenic mechanism is largely unknown. Patients with features of both AIH and PBC are diagnosed with overlap syndrome. The mechanism of the onset of overlap syndrome is completely unknown, and it is currently regarded only as variants of AIH or variants of PBC. We focused on dendritic cells (DCs), which play a central role in both acquired immunity and innate immunity. Antigen presentation for T cell activation is performed on DCs to activate acquired immunity. It has been reported that intracellular vesicle transport plays an important role in antigen presentation. We were interested in what happens to the immune system if the vesicle transport is suppressed in DCs. To solve this question, we generated a gene-deficient mouse that specifically stops the vesicle trans-

port in DCs. Then, all gene-deficient mice died until 41 weeks old. We found that the overlap syndrome characterized by both AIH and PBC developed in the mice. In the lymph nodes of the mice, CD4 T cells were activated and differentiated into Tfh cells and Th1 cells, and in the liver, CD8 T cells were strongly activated and differentiated into cytotoxic T cells expressing granzyme B. In the DCs with abnormal vesicle transport, the expression of major histocompatibility complex (MHC) was significantly enhanced, and the delayed degradation of MHC enhanced the antigen presentation to CD8 T cells. From now on, we plan to elucidate the onset mechanism of this overlap syndrome.

2. Nucleosides drive histiocytosis in SLC29A3 disorders by activating TLR7

Takuma Shibata[1], Masato Taoka[2], Shin-Ichiroh Saitoh[1], Yoshio Yamauchi[2], Yuji Motoi[1], Mayumi Komine[4], Etsuko Fujita[4], Ryota Sato[1], Hiroshi Sagara[3], Takeshi Ichinohe[5], Mimi Kawazoe[1], Chiharu Kato[1], Katsuhiro Furusho[1], Yusuke Murakami[1], Ryutaro Fukui[1], Mamitaro Ohtsuki[4], Umeharu Ohto[6], Toshiyuki Shimizu[6], Nobuaki Yoshida[7], Toshiaki Isobe[2], Kensuke Miyake[1]

[1]Division of Innate Immunity, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan. [2]Department of Chemistry, Graduate School of Science, Tokyo Metropolitan University, Minami-osawa 1-1, Hachioji, Tokyo 192-0397, Japan. [3]Medical Proteomics Laboratory, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan. [4]Department of Dermatology, Jichi Medical University, 3311-1 Yakushiji, Shimotsuke, Tochigi 329-0498, Japan. [5]Division of Viral Infection, Department of Infectious Disease Control, International Research Center for Infectious Diseases, Institute of Medical Science, The University of Tokyo, Minato-ku, Tokyo 108-8639, Japan.[6]Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. [7]Laboratory of Developmental Genetics, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan.

A lysosomal transmembrane protein SLC29A3 transports nucleosides from lysosomes to the cytoplasm. Loss-of-function mutations of the SLC29A3 gene cause lysosomal nucleoside storage in monocyte/macrophages, leading to their accumulation called histiocytosis in humans and mice. Little is known, however, about a mechanism behind nucleoside-dependent histiocytosis. TLR7, an innate immune sensors for single stranded RNA, bind and respond to nucleosides. We here show that they drive nucleoside-mediated histiocytosis. Patrolling monocyte/macrophages accumulate in the spleen of Slc29a3–/– mice but not Slc29a3–/– Tlr7–/– mice. Accumulated patrolling monocyte/macrophages stored nucleosides derived from cell corpse. TLR7 was recruited to phagosomes and activated as evidenced by TLR7-dependent phagosomal maturation. TLR7 induced hyper-responsiveness to M-CSF in Slc29a3–/– monocyte/macrophages. These results suggest that TLR7 drives histiocytosis in SLC29A3 disorders.

3. Anti-TLR7 antibody protects against lupus nephritis in NZBWF1 mice by targeting B cells and patrolling monocytes

Yusuke Murakami[1, 2], Ryutaro Fukui[1], Reika Tanaka[1], Yuji Motoi[1], Atsuo Kanno[1], Ryota Sato[1], Kiyoshi Yamaguchi[3], Hirofumi Amano[4], Yoichi Furukawa[3], Hitoshi Suzuki[5], Yusuke Suzuki[5], Naoto Tamura[4], Naomi Yamashita[2], Kensuke Miyake[1, 6, *]

: [1]Division of Innate Immunity, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo. [2]Department of Pharmacotherapy, Research Institute of Pharmaceutical Sciences, Musashino University. [3]Division of Clinical Genome Research, Advanced Clinical Research Center, The Institute of Medical Science, The University of Tokyo. [4] Department of Internal Medicine and Rheumatology, Juntendo University School of Medicine. [5]Department of Nephrology, Juntendo University Faculty of Medicine. [6]Laboratory of Innate Immunity, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo.

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by autoantibody production and multiple organ damage. Toll-like receptor 7 (TLR7), an innate immune RNA sensor expressed in monocytes/macrophages, dendritic cells (DCs), and B cells, promotes disease progression. However, little is known about the cellular mechanisms through which TLR7 drives lupus nephritis. Here, we show that the anti-mouse TLR7 mAb, but not anti-TLR9 mAb, protected lupus-prone NZBWF1 mice from nephritis. The anti-TLR7 mAb reduced IgG deposition in glomeruli by inhibiting the production of autoantibodies to the RNA-associated antigens. We found a disease-associated increase in Ly6C^{low} patrolling monocytes that expressed high levels of TLR7 and had upregulated expression of lupus-associated molecules in NZBWF1 mice. Anti-TLR7 mAb abolished this lupus-associated increase in patrolling monocytes in the circulation, spleen, and glomeruli. These results suggested that TLR7 drives autoantibody production and lupus-associated monocytosis in NZBWF1 mice and, that anti-TLR7 mAb is a promising therapeutic tool targeting B cells and monocytes/macrophages.

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Department of Microbiology and Immunology

Division of Molecular Virology
ウイルス病態制御分野

Professor	Yasushi Kawaguchi, D.V.M., Ph.D.	教　授	博士(獣医学)　川　口　　寧
Associate Professor	Akihisa Kato, Ph.D.	准教授	博士(医学)　加　藤　哲　久
Assistant Professor	Naoto Koyanagi, Ph.D.	助　教	博士(生命科学)　小　柳　直　人
Assistant Professor	Yuhei Maruzuru, Ph.D.	助　教	博士(生命科学)　丸　鶴　雄　平

In our laboratory, we are promoting strategic fundamental research aimed at developing a novel method of viral infection control by elucidating the mechanism underlying viral proliferation/pathology. Moreover, using viruses as a biological probe, we are also challenging next-generation virology to reconsider viruses as a homeostasis factor and explore their significance, in addition to unraveling cells and physiological control mechanisms that cannot be elucidated by research on normal human hosts.

1. Role of the DNA binding activity of herpes simplex virus 1 VP22 in evading AIM2-dependent inflammasome activation induced by the virus

Yuhei Maruzuru, Naoto Koyanagi, Akihisa Kato, and Yasushi Kawaguchi

AIM2 is a cytosolic DNA sensor of the inflammasome, which induces critical innate immune responses against various invading pathogens. Earlier biochemical studies showed that the binding of AIM2 to DNA triggered the self-oligomerization of AIM2, which is essential for AIM2 inflammasome activation. We recently reported that VP22, a virion tegument protein of herpes simplex virus 1 (HSV-1), inhibited activation of the AIM2 inflammasome in HSV-1-infected cells by preventing AIM2 oligomerization. VP22 binds non-specifically to DNA; however, its role in HSV-1 replication is unclear. We investigated the role of VP22 DNA binding activity in the VP22-mediated inhibition of AIM2 inflammasome activation. We identified a VP22 domain encoded by amino acids 227 to 258 as the minimal domain required for its binding to DNA in vitro Consecutive alanine substitutions in this domain substantially impaired the DNA binding activity of VP22 in vitro and attenuated the inhibitory effect of VP22 on AIM2 inflammasome activation in an AIM2 inflammasome reconstitution system. The inhibitory effect of VP22 on AIM2 inflammasome activation was completely abolished in macrophages infected with a recombinant virus harboring VP22 with one of the consecutive alanine substitutions, similar to the effect of a VP22-null mutant virus. These results suggested that the DNA binding activity of VP22 is critical for VP22-mediated AIM2 inflammasome activation in HSV1-infected cells.

IMPORTANCE VP22, a major component of the HSV-1 virion tegument, is conserved in alphaherpesviruses and has structural similarity to ORF52, a component of the virion tegument that is well-conserved in gammaherpesviruses. Although the potential DNA binding activity of VP22 was discovered decades ago, its significance in the HSV-1 life cycle is poorly understood. Here, we show that the DNA binding activity of VP22 is critical for the inhibition of AIM2 inflammasome activation induced in HSV-1-infected cells. This is the first report to show a role for the DNA binding activity of VP22 in the HSV-1 life cycle, allowing the virus to evade AIM2 inflammasome activation, which is critical for its replication in vivo.

2. Prohibitin-1 contributes to the cell-to-cell transmission of herpes simplex virus 1 via the MAPK/ERK signaling pathway

Mizuki Watanabe, Jun Arii[1], Kosuke Takeshima, Ayano Fukui, Masayuki Shimojima[2], Hiroko Kozuka-Hata[3], Masaaki Oyama[3], Takeharu Minamitani[4], Teruhito Yasui[4], Yuji Kubota[5], Mutsuhiro Takekawa[5], Isao Kosugi[6], Yuhei Maruzuru, Naoto Koyanagi, Akihisa Kato, Yasuko Mori[1], and Yasushi Kawaguchi: [1]Division of Clinical Virology, Center for Infectious Diseases, Kobe University Graduate School of Medicine, Kobe, Hyogo [2]Department of Virology I, National Institute of Infectious Diseases, Tokyo [3]Medical Proteomics Laboratory, The Institute of Medical Science, The University of Tokyo [4]Laboratory of Infectious Diseases and Immunity, National Institutes of Biomedical Innovation, Health, and Nutrition, Ibaraki, Osaka [5]Division of Cell Signaling and Molecular Medicine, Department of Basic Medical Sciences, Institute of Medical Science, The University of Tokyo [6]Department of Regenerative and Infectious Pathology, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka

Viral cell-to-cell spread, a method employed by several viral families for entrance via cell junctions, is highly relevant to the pathogenesis of various viral infections. Cell-to-cell spread of herpes simplex virus 1 (HSV-1) is known to depend greatly on envelope glycoprotein E (gE). However, the molecular mechanism by which gE acts in HSV-1 cell-to-cell spread and the mechanisms of cell-to-cell spread by other herpesviruses remain poorly understood. Here, we describe our identification of prohibitin-1 as a novel gE-interacting host cell protein. Ectopic expression of prohibitin-1 increased gE-dependent HSV-1 cell-to-cell spread. As observed with the gE-null mutation, decreased expression or pharmacological inhibition of prohibitin-1 reduced HSV-1 cell-to-cell spread without affecting the yield of virus progeny. Similar effects were produced by pharmacological inhibition of the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway, wherein prohibitin-1 acts as a protein scaffold and is required for induction of this pathway. Furthermore, artificial activation of the MAPK/ERK pathway restored HSV-1 cell-to-cell spread impaired by the gE-null mutation. Notably, pharmacological inhibition of prohibitins or the MAPK/ERK pathway reduced viral cell-to-cell spread of representative members in all herpesvirus subfamilies. Our results suggest that prohibitin-1 contributes to gE-dependent HSV-1 cell-to-cell spread via the MAPK/ERK pathway and that this mechanism is conserved throughout the Herpesviridae, whereas gE is conserved only in the Alphaherpesvirinae subfamily.

IMPORTANCE Herpesviruses are ubiquitous pathogens of various animals, including humans. These viruses primarily pass through cell junctions to spread to uninfected cells. This method of cell-to-cell spread is an important pathogenic characteristic of these viruses. Here, we show that the host cell protein prohibitin-1 contributes to HSV-1 cell-to-cell spread via a downstream intracellular signaling cascade, the MAPK/ERK pathway. We also demonstrate that the role of the prohibitin-1-mediated MAPK/ERK pathway in viral cell-to-cell spread is conserved in representative members of every herpesvirus subfamily. This study has revealed a common molecular mechanism of the cell-to-cell spread of herpesviruses.

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Department of Microbiology and Immunology

Division of Vaccine Science
ワクチン科学分野

Professor	Ken Ishii, M.D., Ph.D.		教　授	博士(医学)	石　井　　健
Associate Professor	Kouji Kobiyama, Ph.D.		准教授	博士(医学)	小檜山　康　司
Project Senior Assistant Professor	Hideo Negishi, Ph.D.		特任講師	博士(医学)	根　岸　英　雄
Assistant Professor	Burcu Temizoz, Ph.D.		助教	博士(医学)	テミズオズ　ブルジュ

Primary goal of our laboratory is to understand the immunological mechanisms of the intra- and inter-cellular signaling pathways that mediate the immunogenicity of successful vaccines, as well as biological responses to adjuvants. Such knowledge will enable us to develop novel concepts, modalities and next generation immunopreventive and/or therapeutic agents against infectious diseases, cancer and allergy as well as other non-communicable diseases.

1. *Primary Cilia in the Skin: Functions in Immunity and Therapeutic Potential*

The skin is the biggest organ and provides a physical and immunological barrier against pathogen infection. The distribution of primary cilia in the skin of mice has been reported, but which cells in human skin have them has not, and we still know very little about how they change in response to immune reactions or disease. This review introduces several studies that describe mechanisms of cilia regulation by immune reaction and the physiological relevance of cilia regulating proliferation and differentiation of stroma cells, including skin-resident Langerhans cells. We discuss the possibility of primary cilia pathology in allergic atopic dermatitis and the potential for therapies targeting primary cilia signaling.

2. *Increase in primary cilia in the epidermis of patients with atopic dermatitis and psoriasis.*

Primary cilia influence cell activity, and thus have a unique role in maintaining cell proliferation and differentiation. In atopic dermatitis (AD) and psoriasis, areas of skin inflammation exhibit dysregulated keratinocyte homeostasis. The role of primary cilia in these conditions remains unclear. The objectives of this study is to elucidate the incidence of primary cilia in skin inflammation and the potential mechanism underlying the dysregulation of keratinocytes. Primary cilia were observed using immunofluorescence staining. Normal skin samples were compared with skin samples from patients with AD or psoriasis in terms of cilia numbers and length. The effect of cytokine stimulation on ciliogenesis in keratinocytes was analysed using a primary keratinocyte culture. IFT88, an important ciliary intraflagellar protein, was blocked in Th2 and Th17 cytokines-stimulated keratinocytes. These effects were analysed with quantitative polymerase chain reaction and Western blot. Significant increases in ciliated cells were observed in AD and psoriasis skin samples compared with normal skin samples. The stimulation of keratinocytes using Th2 and Th17 cytokines modulated the formation of primary cilia. The amount of IFT88 in the primary cilia associated with the phosphorylation of JNK, but not p38, in keratinocytes stimulated with interleukin-13, 17A and 22. An increase of ciliated cells in the epidermis may impair keratinocyte differentiation under stress conditions caused by inflammation in both AD and psoriasis patients.

3. Type I and II interferons toward ideal vaccine and immunotherapy

Introduction: Innate immunity is armed with interferons (IFNs) that link innate immunity to adaptive immunity to generate long-term and protective immune responses against invading pathogens and tumors. However, regulation of IFN production is crucial because chronic IFN responses can have deleterious effects on both antitumor and antimicrobial immunity in addition to provoking autoinflammatory or autoimmune conditions.Areas covered: Here, we focus on the accumulated evidence on antimicrobial and antitumor activities of type I and II IFNs. We first summarize the intracellular and intercellular mechanisms regulating IFN production and signaling. Then, we discuss the mechanisms modulating the dual nature of IFNs for both antitumor and antimicrobial immune responses. Finally, we review the detrimental role of IFNs for induction of autoinflammation and autoimmunity.Expert opinion: The current evidence suggests that the dual role of IFNs for antimicrobial and antitumor immunity is dependent not only on the timing, administration route, and dose of IFNs but also on the type of pathogen/tumor. Therefore, we think that combinatorial therapies involving IFN-inducing adjuvants and immune-checkpoint blockers may offer therapeutic potential, especially for cancer, whereas infectious, autoinflammatory or autoimmune diseases require fine adjustment of timing, dose, and route of the administration for candidate IFN-based vaccines or immunotherapies.

4. A CpG Oligonucleotide Annealed to a Complementary Strand With an Amphiphilic Chain Unit, Acts as a Potent Cancer Vaccine Adjuvant by Targeting Draining Lymph Nodes

Robust induction of cancer-antigen-specific CD8+ T cells is essential for the success of cancer peptide vaccines, which are composed of a peptide derived from a cancer-specific antigen and an immune-potentiating adjuvant, such as a Toll-like receptor (TLR) agonist. Efficient delivery of a vaccine antigen and an adjuvant to antigen-presenting cells in the draining lymph nodes (LNs) holds key to maximize vaccine efficacy. Here, we developed S-540956, a novel TLR9-agonistic adjuvant consisting of B-type CpG ODN2006 (also known as CpG7909), annealed to its complementary sequence oligodeoxynucleotide (ODN) conjugated to a lipid; it could target both a cancer peptide antigen and a CpG-adjuvant in the draining LNs. S-540956 accumulation in the draining LNs and activation of plasmacytoid dendritic cells (pDCs) were significantly higher than that of ODN2006. Mechanistic analysis revealed that S-540956 enhanced the induction of MHC class I peptide-specific CD8+ T cell responses via TLR9 in a CD4+ T cell-independent manner. In mice, the therapeutic effect of S-540956-adjuvanted with a human papillomavirus (HPV)-E7 peptide vaccine against HPV-E7-expressing TC-1 tumors was significantly better than that of an ODN2006-adjuvanted vaccine. Our findings demonstrate a novel adjuvant discovery with the complementary strand conjugated to a lipid, which enabled draining LN targeting and increased ODN2006 accumulation in draining LNs, thereby enhancing the adjuvant effect. Our findings imply that S-540956 is a promising adjuvant for cancer peptide vaccines and has a high potential for applications in various vaccines, including recombinant protein vaccines.

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Department of Microbiology and Immunology

Division of Malaria Immunology
マラリア免疫学分野

Professor	Cevayir Coban, M.D., Ph.D. (Clinical Microbiology)	教　授	博士(医学)(臨床微生物学位)　チョバン　ジェヴァイア
Project Assistant Professor	Michelle S.J. Lee, Ph.D.	特任助教	博士(医学)　リー　ミシェル

Malaria, caused by Plasmodium parasites, often leads to severe complications such as cerebral malaria and death. On the other hand, majority of people recover from malaria, but suffer from partial, but not complete, immunity against parasites. In our lab, we elucidate the mechanisms involved in the host immune system and Plasmodium parasites interactions. Our ultimate goal is to develop vaccines and vaccine modalities against malaria and other infectious diseases.

1. B cell intrinsic TBK1 is essential for germinal center formation during infection and vaccination in mice

Michelle S. J. Lee[1,2], Takeshi Inoue[3], Wataru Ise[3], Julia Matsuo-Dapaah[1], James B. Wing[4,5], Burcu Temizoz[6,2], Kouji Kobiyama[6,2], Tomoya Hayashi[6,2], Ashwini Patil[7], Shimon Sakaguchi[8], A. Katharina Simon[9], Jelena S. Bezbradica[9], Satoru Nagatoishi[10], Kouhei Tsumoto[10,11], Jun-Ichiro Inoue[10], Shizuo Akira[12], Tomohiro Kurosaki[3], Ken J. Ishii[6,12,2], Cevayir Coban[1,12,2]: [1]Division of Malaria Immunology, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan, [2]International Vaccine Design Center, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan, [3]Laboratory of Lymphocyte Differentiation, Immunology Frontier Research Center, Osaka University, Osaka, Japan, [4]Laboratory of Human Immunology (Single Cell Immunology), Immunology Frontier Research Center, Osaka University, Osaka, Japan, [5]Human Single Cell Immunology Team, Center for Infectious Disease Education and Research, Osaka University, Osaka, Japan, [6]Division of Vaccine Science, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan, [7]Combinatics Inc., Tokyo, Japan, [8]Laboratory of Experimental Immunology, Immunology Frontier Research Center, Osaka University, Osaka, Japan, [9]The Kennedy Institute of Rheumatology, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Oxford, UK, [10]Research Platform Office, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan, [11]Department of Bioengineering, Graduate School of Engineering, The University of Tokyo, Tokyo, Japan, [12]Immunology Frontier Research Center, Osaka University, Osaka, Japan.

The germinal center (GC) is a site where somatic hypermutation and clonal selection are coupled for antibody affinity maturation against infections. However, how GCs are formed and regulated is incompletely understood. Here, we identified an unexpected role of Tank-binding kinase-1 (TBK1) as a crucial B cell-intrinsic factor for GC formation. Using immunization and malaria infection models, we show that TBK1-deficient B cells failed to form GC despite normal Tfh cell differentiation, although some malaria-infected B cell-specific TBK1-deficient mice could survive by GC-independent mechanisms. Mechanistically, TBK1 phosphorylation elevates in B cells during GC differentiation and regulates the balance of IRF4/BCL6 expression by limiting CD40 and BCR activation through noncanonical NF-κB and AKTT308

signaling. In the absence of TBK1, CD40 and BCR signaling synergistically enhanced IRF4 expression in Pre-GC, leading to BCL6 suppression, and therefore failed to form GCs. As a result, memory B cells generated from TBK1-deficient B cells fail to confer sterile immunity upon reinfection, suggesting that TBK1 determines B cell fate to promote long-lasting humoral immunity.

2. Using a new three-dimensional CUBIC tissue-clearing method to examine the brain during experimental cerebral malaria

Julia Matsuo-Dapaah[1,2] , Michelle Sue Jann Lee[1], Ken J Ishii [2,3,4,5], Kazuki Tainaka[6,7], Cevayir Coban[1,2,4,5]: [1]Division of Malaria Immunology, Department of Microbiology and Immunology, Institute of Medical Science (IMSUT), University of Tokyo, Tokyo, Japan, [2]Graduate School of Medicine, University of Tokyo, Tokyo, Japan, [3]Division of Vaccine Science, Department of Microbiology and Immunology, Institute of Medical Science (IMSUT), University of Tokyo, Tokyo, Japan, [4]International Vaccine Design Center, Institute of Medical Science (IMSUT), University of Tokyo, Tokyo, Japan, [5]Immunology Frontier Research Center (IFReC), Osaka University, Osaka, Japan, [6]Department of System Pathology for Neurological Disorders, Center for Bioresources, Brain Research Institute, Niigata University, Niigata, Japan, [7]Laboratory for Synthetic Biology, RIKEN Center for Biosystems Dynamics Research, Osaka, Japan.
of Malaria Immunology.

Cerebral malaria (CM) is a life-threatening complication of the malaria disease caused by Plasmodium falciparum infection and is responsible for the death of half a million people annually. The molecular pathogenesis underlying CM in humans is not completely understood, although sequestration of infected erythrocytes in cerebral microvessels is thought to play a major role. In contrast, experimental cerebral malaria (ECM) models in mice have been thought to be distinct from human CM, and are mainly caused by inflammatory mediators. Here, to understand the spatial distribution and the potential sequestration of parasites in the whole-brain microvessels during a mouse model of ECM, we utilized the new tissue-clearing method CUBIC (Clear, Unobstructed, Brain/Body Imaging Cocktails and Computational analysis) with light-sheet fluorescent microscopy (LSFM), and reconstructed images in three dimensions (3D). We demonstrated significantly greater accumulation of Plasmodium berghei ANKA (PbANKA) parasites in the olfactory bulb (OB) of mice, compared with the other parts of the brain, including the cerebral cortex, cerebellum and brainstem. Furthermore, we show that PbANKA parasites preferentially accumulate in the brainstem when the OB is surgically removed. This study therefore not only highlights a successful application of CUBIC tissue-clearing technology to visualize the whole brain and its microvessels during ECM, but it also shows CUBIC's future potential for visualizing pathological events in the whole ECM brain at the cellular level, an achievement that would greatly advance our understanding of human cerebral malaria.

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Lee MSJ, Inoue T, Ise W, Matsuo-Dapaah J, Wing JB, Temizoz B, Kobiyama K, Hayashi T, Patil A, Sakaguchi S, Simon AK, Bezbradica JS, Nagatoishi S, Tsumoto K, Inoue JI, Akira S, Kurosaki T, Ishii KJ, Coban C. B cell intrinsic TBK1 is essential for germinal center formation during infection and vaccination in mice. Journal of Experimental Medicine, Feb 7;219(2):e20211336, 2022.

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Department of Cancer Biology

Division of Molecular Pathology
人癌病因遺伝子分野

Professor	Yoshinori Murakami, M.D., Ph.D.	教　授	医学博士	村　上　善　則
Assistant Professor	Takeshi Ito, Ph.D.	助　教	博士(医学)	伊　東　　剛
Project Assistant Professor	Masaru Koido, Ph.D.	特任助教	博士(工学)	小井土　　大

Elucidation of genetic and epigenetic alterations is essential to provide molecular targets for prevention, diagnosis, and treatment of human cancer. Our current interest is to understand the roles of cell-cell interaction in invasion, metastasis, drug resistance and immunological responses of cancer. Genomic abnormalities involved in human tumors, including lung, breast, thyroid, head and neck cancer, cholangiocarcinoma, adult T-cell leukemia, as well as mechanisms of and genetic susceptibility to various diseases are being investigated.

1. The biological functions of cell-cell interaction in human oncogenesis

Takeshi Ito, Yumi Tsuboi, Yutaka Kasai, Toko Funaki, Yuki Azuma, Yoshiaki Kanamoto, Yue Guo, Mizuki Tominaga, Miko Komiya, Yuya Sohinohara, Kaito Akiyama , Jialiang Nie, Tomoko Masuda, Hiromi Ichihara, Motoi Oba, Zenichi Tanei[1], Akiteru Goto[2], Daisuke Matsubara[3] and Yoshinori Murakami; [1]Department of Cancer Pathology, Faculty of Medicine, Hokkaido University, Sapporo, [2]Department of Diagnostic Pathology, University of Tsukuba, Tsukuba, [3]Cellular and Organ Pathology, Graduate School of Medicine, Akita University, Akita

Disruption of cell adhesion is a critical step to invasion and metastasis of human cancer and their acquired resistance to several anti-cancer and molecular targeting drugs. CADM1/TSLC is an immunoglobulin superfamily cell adhesion molecule (IgCAMs) and acts as a tumor suppressor in various epithelial cancers. By contrast, CADM1 promotes cell invasion and metastasis in adult T-cell leukemia (ATL) or small cell lung cancer (SCLC). We are currently investigating molecular pathways of CADM1 underlying its dual roles in oncogenesis.

In this year, we investigated possible oncogenic roles of CADM1 in SCLC. CADM1 is highly expressed in SCLC and promotes colony formation of SCLC cells in soft agar. We found that one of the 4.1-binding proteins, 4.1R, was responsible for suppression of colony formation by CADM1 in SCLC cells. In primary SCLC, CADM1 expression was correlated with membranous localization of 4.1R and was associated with more advanced tumor stage. These results suggest that the formation of CADM1–4.1R complex would promote malignant features of SCLC (1).

Next, we investigated a role of CADM1 in ATL because CADM1 is overexpressed in ATL and provides a cell-surface diagnostic marker. CADM1 promotes adhesion of ATL cells to vascular endothelial cells and multiple organ infiltration in mice. We show that CADM1 enhances liver infiltration of mouse T-cell lymphoma cells, EL4, after tail vein injection, whereas a CADM1 mutant lacking adhesive activity did not. Furthermore, CADM1-mediated liver infiltration of EL4 cells was canceled in conventional and vascular endothelium-specific *Cadm1* knockout mice, whereas it was not canceled in *Cadm4* knockout mice, which is

another candidate of CADM1 interacting protein. These results suggest that CADM1 on host vascular endothelial cells is required for organ infiltration of ATL and other T-cell lymphomas expressing CADM1 (19).

We are also investigating possible crosstalk of IgCAMs and its biological and immunological significance comprehensively by cloning more than 300 IgCAMs expressed in human cells and analyzing molecule-molecule interactions using the surface plasmon resonance imaging (SPRi) and the amplified luminescence proximity homogenous assay (ALPHA). Significant interaction was then evaluated individually using biological assays between molecule to cell, cell to cell and cell to tissue generated in our laboratory. We have identified several candidate IgSFs involved in cancer metastasis and tumor immune-checkpoint regulation.

2. Studies for establishing novel diagnostic and therapeutic approaches to small cell lung cancer and several human cancer

Takeshi Ito, Motoi Oba, Tomoko Masuda, Zenichi Tanei[1], Daisuke Matsubara[3] and Yoshinori Murakami:

CADM1 is overexpressed in adult T-cell leukemia (ATL) and small cell lung cancer (SCLC), conferring invasive or metastatic phenotypes characteristic to ATL or SCLC. Interestingly, SCLC expresses a splicing variant of CADM1v8/9 containing a unique juxta-membrane fragment, which is specific to normal testis and SCLC. Since CADM1v8/9 fragments are digested by protease and released into blood stream, this fragment could provide a novel serum marker of SCLC. Thus, to establish a sensitive and specific serum marker for diagnosis of SCLC, monoclonal antibodies against the fragments of CADM1v8/9 have been generated and characterized (PCT/JP2019/011201). Antibodies against the O-glycosylated fragment of CADM1v8/9 are being generated and characterized using mass spectrometry analysis of CADM1v8/9 fragments expressed in SCLC cells. These detection systems of SCLC are being validated using the serum from SCLC patients in collaboration with clinical oncologists in the University of Tokyo Hospital and National Cancer Center Hospital. Additional antibodies against CADM1 are being tested for their anti-tumor activity against SCLC by radioisotope- or drug-conjugated antibodies.

3. Analyses of genomic and epigenomic alterations of human lung, breast, head and neck and other cancers.

Ayaka Sato, Ken Akashi, Fumi Murakami, Takeshi Ito, Diasuke Matsubara[3], and Yoshinori Murakami:

To unveil additional molecular mechanisms underlying multistage carcinogenesis, genomic, epigenomic, and transcriptional alterations in key molecules in human tumorigenesis were examined in various cancers.

We examined somatic alterations at sites of CNV in DNA from 92 breast cancers using a CNV-specific Comparative Genomic Hybridization array with 412,000 sites or quantitative PCR analysis. Somatic copy number alterations (CNAs) were detected in 39.9% of the CNV probes examined. When CNA fragments were categorized according to their size, CNAs smaller than 10kb correlated significantly with ER-positivity and lower NG, providing a novel mechanism in ER-positive breast carcinogenesis (2).

Next, we examined the clinical significance of circulating tumor DNA in primary papillary thyroid cancer (PTC) and oropharyngeal cancer. In PTC, we examined the significance of ctDNA carrying the *BRAF* V600E mutation. Twenty-two primary PTC patients without distant metastasis were enrolled and the presence or absence of the *BRAF* V600E mutation was examined by droplet digital PCR (ddPCR). The *BRAF* V600E mutation was detected in 16 of 22 (73%) primary tumors. Five of 16 (31%) cases carrying the *BRAF* V600E mutation in their tumors showed the identical mutation in pre-surgery plasma. Extra-thyroidal extension of the primary tumor correlated significantly with the *BRAF* V600E mutation in pre-surgery ctDNA (P=0.025). Moreover, one patient with the mutated *BRAF* V600E in the primary tumor showed the identical mutation not in pre-surgery ctDNA but in post-surgery ctDNA. This patient had regional lymph node recurrence six months after surgery. These results suggest that detection of the *BRAF* V600E mutation in pre-surgery plasma can predict local progression of the primary tumor and that the increased fractional abundance of the mutated *BRAF* V600E in post-surgery ctDNA might predict PTC recurrence (3).

In papillomavirus-related p16-positive oropharyngeal cancer, we validated the significance of ctDNA of Human Papilloma Virus (HPV)-derived sequences as a biomarker. We assessed 25 patients with p16-positive oropharyngeal cancer. The ctDNA was extracted from the plasma and analyzed using digital polymerase chain reaction. HPV-derived ctDNA was detected in 14 (56%) of the 25 patients. In all patients, the samples were found to be ctDNA-negative after initial treatment. Cancer recurrence was observed in 2 of the 14 patients, and HPV-derived ctDNA was detected in their blood at the time of recurrence. Our results indicate that HPV-derived ctDNA is a prospective biomarker for predicting the recurrence of p16-positive oropharyngeal cancer (4).

Furthermore, molecular and genetic mechanisms of clonal hematopoiesis (5) hereditary predisposition to cancer (20) and cancer progression through Mint3–FIH-1 cascade in response to hypoxia and possible

application of its pharmacological inhibition (6) were also analyzed in collaboration with others.

4. Genomic-epidemiological studies of various human diseases and phenotypes using the materials and information of Biobank Japan.

Masaru Koido, Takayuki Morisaki, Yoshinori Murakami, Yoichiro Kamatani[4] and Koichi Matsuda; [4]Laboratory of Complex Trait Genomics and [5]Laboratory of Clinical Genome Sequencing, Graduate School of Frontier Sciences, The University of Tokyo

To reveal the molecular mechanisms of complex diseases and various phenotypes, we carried out genome-wide association studies (GWAS) and their downstream analysis for steroid-associated osteonecrosis of the femoral head in systemic lupus erythematosus (7), colorectal polyps (8), inguinal hernias (9), atopic dermatitis (10) and lipid metabolism (11), as well as 220 phenotypes of individuals (12). Among these studies, we identified putative causal variants and their target transcripts for inguinal hernias (9) and atopic dermatitis (10) using our machine learning pipeline MENTR (mutation effect prediction on ncRNA transcription; Koido *et al., bioRxiv.* 2020). We also participated in several post-GWAS studies, for example, development of polygenic risk score for adolescent idiopathic scoliosis (13), and Mendelian randomization study showing evidence that genetically high circulating FUT3 levels can reduce the risk of idiopathic pulmonary fibrosis (14). To promote such genetic studies for preventing and treating complex diseases in academia and company worldwide, we released genotype data for >54,000 patients from BioBank Japan (https://humandbs.biosciencedbc.jp/en/hum0311-v1) and prepared to release serum metabolome data from a collaboration study between Nightingale Health Japan and BioBank Japan.

For single gene disorders, patients and family members with genetic vascular diseases, including connective tissue disorders like Marfan syndrome, Loeys-Dietz syndrome (15, 16) and related diseases (17, 18) were diagnosed by by taking their history, physical examination, imaging including echo-cardiography and genetic examination, though some patients were not clarified regarding their pathogenic genes. Study to identify novel pathogenic genes for genetic vascular diseases was being performed.

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Department of Cancer Biology

Division of Genetics
腫瘍抑制分野

Professor	Yuji Yamanashi, Ph.D.	教　授	理学博士　山　梨　裕　司
Assistant Professor	Ryo Ueta, Ph.D.	助　教	博士（生命科学）　植　田　　亮
Assistant Professor	Akane Inoue-Yamauchi, Ph.D.	助　教	博士（医学）　山内（井上）茜
Assistant Professor	Takahiro Eguchi, Ph.D.	助　教	博士（科学）　江　口　貴　大
Assistant Professor	Maria Tsoumpra, Ph.D.	助　教	博士（分子薬理学）　ツゥオムプラ　マリア

The major interest of this division is in molecular signals that regulate a variety of cellular activities. Our aim is to address how dysregulated cellular signals give rise to neoplastic, immune, neural, metabolic, or developmental disorders. Our goal is to understand the molecular bases of tumorigenesis and the development of other intractable diseases as a path toward uncovering therapeutic targets. Currently, we are investigating regulatory mechanisms in protein-tyrosine kinase (PTK)-mediated signaling pathways, their pathophysiological roles and the potential for therapeutic intervention.

1. Activation of the receptor tyrosine kinase MuSK by the cytoplasmic protein Dok-7 in neuromuscular synaptogenesis.

Eguchi, T., Tokuoka, T., Yoda, M., Ueta, R., Tezuka, T.[1], Weatherbee, SD.[2], Watanabe, Y.[3], Sagara, H.[3], Nagatoishi, S.[3], Tsumoto, K.[3], and Yamanashi, Y.: [1] Present affiliation: Center for the Promotion of Interdisciplinary Education and Research, Kyoto University. [2]Department of Genetics, Yale University. [3]Medical Proteomics Laboratory, IMSUT.

Protein-tyrosine kinases (PTKs) play crucial roles in a variety of signaling pathways that regulate proliferation, differentiation, motility, and other activities of cells. Therefore, dysregulated PTK signals give rise to a wide range of diseases such as neoplastic disorders. To understand the molecular bases of PTK-mediated signaling pathways, we identified Dok-1 as a common substrate of many PTKs in 1997. Since then, the Dok-family has been expanded to seven members, Dok-1 to Dok-7, which share structural similarities characterized by N-terminal pleckstrin homology (PH) and phosphotyrosine binding (PTB) domains, followed by Src homology 2 (SH2) target motifs in the C-terminal moiety, suggesting an adaptor function. Indeed, as described below, Dok-1 and Dok-2 recruit p120 rasGAP upon tyrosine phosphorylation to suppress Ras-Erk signaling. However, we found that Dok-7 acts as an essential cytoplasmic activator of the muscle-specific receptor tyrosine kinase (RTK) MuSK in the formation and maintenance of the neuromuscular junction (NMJ), providing a new insight into RTK-mediated signaling. It seems possible that local levels of cytoplasmic activators, like Dok-7, control the activity of RTKs in concert with their extracellular ligands.

The NMJ is a synapse between a motor neuron and skeletal muscle, where the motor nerve terminal is apposed to the endplate (the region of synaptic specialization on the muscle). The contraction of skeletal muscle is controlled by the neurotransmitter acetylcholine (ACh), which is released from the presynaptic motor nerve terminal. To achieve efficient neuromuscular transmission, acetylcholine receptors (AChRs) must be densely clustered on the postsynaptic muscle membrane of the NMJ. Failure of AChR clustering is associated with disorders of neuromuscular transmission such as congenital myasthenic syndromes and myasthenia gravis, which are characterized by fatiga-

ble muscle weakness. The formation of NMJs is orchestrated by MuSK and by neural agrin, an extracellular activator of MuSK. However, experimentally when motor nerves are ablated, AChRs form clusters in the correct, central region of muscle during embryogenesis in a MuSK-dependent process known as prepatterning of the receptors. In addition, in vivo overexpression of MuSK causes neuromuscular synapse formation in the absence of agrin, suggesting that muscle-intrinsic, cell-autonomous activation of MuSK may be adequate to trigger presynaptic and postsynaptic differentiation in vivo. However, the mechanisms by which MuSK is activated independently of nerve and agrin had long been unclear.

Because both MuSK and the adaptor-like cytoplasmic protein Dok-7 are localized to the postsynaptic region of NMJs, we examined their interaction and found that Dok-7 is an essential cytoplasmic activator of MuSK. In addition, we found that Dok-7 directly interacts with the cytoplasmic portion of MuSK and activates the RTK, and that neural agrin requires Dok-7 in order to activate MuSK. Indeed, in vivo overexpression of Dok-7 increased MuSK activation and promoted NMJ formation. Conversely, mice lacking Dok-7 formed neither NMJs nor AChR clusters. In addition, we found that postnatal knockdown of *dok-7* gene expression in mice causes structural defects in NMJs and myasthenic pathology, suggesting an essential role for Dok-7 not only in the embryonic formation but also in the postnatal maintenance of NMJs. Furthermore, we found that forced expression of Dok-7 lacking the C-terminal region rescued Dok-7 knockout mice from neonatal lethality caused by the lack of NMJs, indicating restored MuSK activation and NMJ formation. However, these mice showed only marginal activation of MuSK and died by 3 weeks of age apparently due to an abnormally small number and size of NMJs. Therefore, Dok-7's C-terminal region plays a key, but not fully essential, role in MuSK activation and NMJ formation.

Interestingly, mice lacking Lrp4, which forms a complex with MuSK and acts as an essential agrin-binding module, do not show MuSK-dependent AChR prepatterning or NMJ formation. This suggests that Lrp4 is required for MuSK activation under physiological conditions, in contrast to our observation that Dok-7 can activate MuSK in the absence of Lrp4 or its ligand agrin, at least in vitro. Thus, we examined the effects of forced expression of Dok-7 in skeletal muscle on NMJ formation in the absence of Lrp4 and found that it indeed induces MuSK activation in mice lacking Lrp4. However, the activation level of MuSK was significantly lower in the absence than in the presence of Lrp4. Together, these data indicate that Lrp4 is required for efficient activation of MuSK by Dok-7 in the muscle. Since Lrp4 is also essential for presynaptic differentiation of motor nerve terminals in the embryonic NMJ formation (*Nature* 489:438-442, 2012), this apparent cooperation between Lrp4 and Dok-7 in MuSK activation may be complicated.

The NMJs are cholinergic synapses characterized by ultrastructural specializations, including the presynaptic active zones, the acetylcholine (ACh) release sites of the motor nerve terminal, and the postsynaptic junctional folds of muscle membrane, where ACh receptors (AChRs) cluster in the nearby areas of active zones for efficient neuromuscular transmission. Interestingly, overexpression of Dok-7 in skeletal muscle abnormally activates MuSK, leading to the formation of abnormally large NMJs in mice. However, these mice with abnormally large NMJs show no obvious motor dysfunction. Recently, we have found that Dok-7 overexpression enhances NMJ transmission less markedly than NMJ size. Consistent with this, ultrastructural analyses revealed that the densities of active zones and synaptic vesicles in the presynaptic motor nerve terminals were reduced. In addition, the density and size of postsynaptic junctional folds in the muscle membrane were also reduced. Moreover, terminal Schwann cells (tSCs) exhibits significantly greater penetration of their processes into the synaptic clefts, which connect the pre- and post-synaptic specializations. Together, our findings demonstrate that forced expression of Dok-7 in muscle enhances neuromuscular transmission with significant enlargement and ultrastructural alterations of NMJs, providing implications in robustness of neuromuscular transmission. We are investigating Dok-7-mediated signaling, including downstream effectors, in regulating formation, maintenance and function of NMJs to develop new therapeutic approaches against NMJ disorders.

2. Agrin's role aside from MuSK activation in the postnatal maintenance of NMJs.

Eguchi, T., Tezuka, T., Fan, W., Ochiai, C., Burgess, RW.[1], Ueta, R., and Yamanashi, Y.: [1]The Jackson Laboratory.

Although NMJ formation requires agrin under physiological conditions, it is dispensable for NMJ formation experimentally in the absence of the neurotransmitter acetylcholine, which inhibits postsynaptic specialization. Thus, it was hypothesized that MuSK needs agrin together with Lrp4 and Dok-7 to achieve sufficient activation to surmount inhibition by acetylcholine. To test this hypothesis, we examined the effects of forced expression of Dok-7 in skeletal muscle on NMJ formation in the absence of agrin and found that it indeed restores NMJ formation in agrin-deficient embryos. However, these NMJs rapidly disappeared after birth, whereas exogenous Dok-7-mediated MuSK activation was maintained. These findings indicate that the MuSK activator agrin plays another role essential for the postnatal maintenance, but not for embryonic formation, of NMJs. Because patho-

genic mutations of agrin in patients with congenital myasthenic syndromes (see below) did not show impaired ability to activate MuSK at least in vitro (*Am. J. Hum. Genet.*, 85:155-167, 2009; *JCI Insight*, 5:e132023, 2020), the novel role of agrin may be relevant to pathogenicity of the mutations. We are investigating molecular mechanisms underlying the agrin-mediated postnatal maintenance of NMJs by utilizing mice expressing various forms of agrin mutants.

3. Pathophysiological mechanisms underlying *DOK7* myasthenia.

Eguchi, T., Tezuka, T., Ueta, R., Fukudome, T.[1], Watanabe, Y., Sagara, H., Motomura, M.[2], Beeson, DMW.[3], and Yamanashi, Y.: [1]Department of Neurology, Nagasaki Kawatana Medical Center. [2]Department of Engineering, Faculty of Engineering, Nagasaki Institute of Applied Science. [3]Weatherall Institute of Molecular Medicine, University of Oxford.

As mentioned above, impaired clustering of AChRs could underlie NMJ disorders, be they autoimmune (myasthenia gravis) or genetic (congenital myasthenic syndromes (CMSs)) in origin. Therefore, our findings that Dok-7 activates MuSK to cluster AChRs and to form NMJs suggested *DOK7* as a candidate gene for mutations associated with CMS. Indeed, we demonstrated that biallelic mutations in *DOK7* underlie a major subgroup of CMS with predominantly proximal muscle weakness that did not show tubular aggregates on muscle biopsy but were found to have normal AChR function despite abnormally small and simplified NMJs. We further demonstrated that several mutations, including one associated with the majority of patients with the disease, impaired Dok-7's ability to activate MuSK. This new disease entity is termed "*DOK7* myasthenia."

To investigate pathophysiological mechanisms underlying *DOK7* myasthenia, we established knock-in mice (Dok-7 KI mice) that have a mutation associated with the majority of patients with *DOK7* myasthenia. As expected, Dok-7 KI mice showed characteristic features of severe muscle weakness and died between postnatal day 13 and 20. Furthermore, they showed abnormally small NMJs lacking postsynaptic folding, a pathological feature seen in patients with *DOK7* myasthenia. Consistent with this, Dok-7 KI mice exhibited decreased MuSK activity in skeletal muscle, indicating that the Dok-7 KI mice develop defects similar to those found in patients with *DOK7* myasthenia, although the mice exhibit a more severe phenotype. In collaboration with Prof. David Beeson's group, we examined NMJ functions in the Dok-7 KI mice in the absence or presence of salbutamol, a β2-adrenergic agonist, which is an effective treatment for *DOK7* myasthenia. This study revealed that salbutamol can prolong survival and increase NMJ number in Dok-7 KI mice, suggesting a similar mode of action in patients. We are investigating molecular pathways underlying NMJ defects and muscle weakness in Dok-7 KI mice to develop mechanism-based therapeutic approaches against *DOK7* myasthenia.

4. *DOK7* gene therapy that enlarges NMJs.

Eguchi, T., Ueta, R., Sugita, S.[1], Minegishi, Y.[1], Motomura, M., Beeson, DMW., Shimotoyodome, A.[1], Ota, N.[1], Ogiso, N.[2], Takeda, S.[3], Okada, T.[4], and Yamanashi, Y.: [1]Biological Science Research, Kao Corporation. [2]Laboratory of Experimental Animals, National Center for Geriatrics and Gerontology. [3]Department of Molecular Therapy, National Institute of Neuroscience. [4]Division of Molecular and Medical Genetics, Center for Gene and Cell Therapy, IMSUT

As mentioned above, *DOK7* myasthenia is associated with impaired NMJ formation due to decreased ability of Dok-7 to activate MuSK in myotubes at least in part. Interestingly, in vivo overexpression of Dok-7 increased MuSK activation and promoted NMJ formation in the correct, central region of the skeletal muscle. Because these genetically manipulated mice did not show obvious defects in motor activity, overexpression of Dok-7 in the skeletal muscle of patients with *DOK7* myasthenia might ameliorate NMJ formation and muscle weakness. To test this possibility, we generated an Adeno-associated virus-based vector (AAV-D7), which strongly expressed human Dok-7 in myotubes and induced AChR cluster formation. Indeed, therapeutic administration of AAV-D7 to Dok-7 KI mice described above resulted in enlargement of NMJs and substantial increases in muscle strength and life span. Furthermore, when applied to model mice of another neuromuscular disorder, autosomal dominant Emery-Dreifuss muscular dystrophy, therapeutic administration of AAV-D7 (*DOK7* gene therapy) likewise resulted in enlargement of NMJs as well as positive effects on motor activity and life span. Interestingly, *DOK7* gene therapy suppressed denervation (nerve detachment) at NMJs, and enhanced motor activity and life span in a mouse model of familial amyotrophic lateral sclerosis (ALS), a progressive, multifactorial motor neurodegenerative disease with severe muscle atrophy. These results suggest potential for *DOK7* gene therapy in age-related decline in motor function, where NMJ denervation appears to play a crucial role similar to that observed in ALS model mice. Indeed, we have recently found that *DOK7* gene therapy significantly enhances motor function and muscle strength together with NMJ innervation in aged mice. We are further investigating the effects, including ultrastructural and electrophysiological ones, of AAV-D7 administration in multiple types of muscle weakness, including age-related one.

5. Potential of CaMKIIβ as a novel therapeutic target for enhancing muscle mass and strength.

Eguchi, T., Fan, W., and Yamanashi, Y.

Ca^{2+}/calmodulin-dependent kinase II (CaMKII) is a family of protein serine/threonine kinases known to be an effector of Ca^{2+} signaling. We recently demonstrated that forced expression of inactive form (K43M) of CaMKIIβ, an isoform of CaMKII, by AAV (adeno-associated virus) enhanced muscle mass and strength, demonstrating the potential of CaMKIIβ as a novel therapeutic target to counteract skeletal muscle atrophy. We are currently investigating the effects of AAV-CaMKIIβ-K43M administration in multiple types of muscle atrophy.

6. Roles of Dok-1 to Dok-6.

Inoue-Yamauchi, A., Wu, W., Sato, T., Jozawa, H., Kanno, T., Arimura, S.[1], and Yamanashi, Y.: [1]Present affiliation: Department of Medicine Section of Gastroenterology and Hepatology, Baylor College of Medicine.

Dok-family proteins can be classified into three subgroups based on their structural similarities and expression patterns; namely, 1) Dok-1, -2, and -3, which are preferentially expressed in hematopoietic cells, 2) Dok-4, -5, and -6, which are preferentially expressed in non-hematopoietic cells, and 3) Dok-7, which is preferentially expressed in muscle cells. As mentioned above, Dok-1 and its closest paralog, Dok-2, recruit p120 rasGAP upon tyrosine phosphorylation to suppress Ras-Erk signaling. Although Dok-3 does not bind with p120 rasGAP, it also inhibits Ras-Erk signaling. Consistently, we demonstrated that Dok-1, Dok-2 and Dok-3 are key negative regulators of hematopoietic growth and survival signaling. For example, Dok-1, Dok-2, and Dok-3 cooperatively inhibit macrophage proliferation and *Dok-1*$^{-/-}$Dok-2$^{-/-}$ Dok-3$^{-/-}$ mice develop histiocytic sarcoma, an aggressive malignancy of macrophages. Also, we found that Dok-1 and Dok-2 negatively regulate intestinal inflammation in the dextran sulfate sodium-induced colitis model, apparently through the induction of IL-17A and IL-22 expression. However, we recently found that Dok-3 and Dok-1/-2 play distinctive roles in cell fusion and proliferation during osteoclastogenesis and cooperatively protect mice from osteopenia. We are currently investigating molecular mechanisms underlying Dok-3-mediated suppression of osteoclast fusion, which may lead to developing new therapeutic approaches against disorders associated with osteopenia. Also, we are investigating physiological and pathological roles of Dok-1 to Dok-6, including those in, tumor malignancy, inflammatory disorders and tissue injury.

7. Roles of C/EBPδ.

Inoue-Yamauchi, A., Jozawa, H., Wu, W., and Yamanashi, Y.

The CCAT/enhancer-binding protein delta (C/EBPδ), a basic leucine zipper transcription factor, regulates many biological processes such as inflammation, cell proliferation, differentiation and genomic stability. We recently demonstrated that C/EBPδ plays an essential role in suppressing dextran sulfate sodium-induced colitis, likely by attenuating intestinal epithelial cell apoptosis. We are further investigating pathological roles of C/EBPδ and its homologue C/EBPβ in inflammatory diseases, including studies with tissue-specific gene manipulation.

8. Omic analyses.

Eguchi, T., Jozawa, H., Fan, W., Tokuoka, Y., Yoda, M., Wu, W., Ueta, R., Iemura, S.[1], Natsume, T.[2], Kozuka-Hata, H.[3], Oyama, M.[3], and Yamanashi, Y.: [1]Translational Research Center, Fukushima Medical University. [2]National Institute of Advanced Science and Technology, Molecular Profiling Research Center for Drug Discovery. [3]Medical Proteomics Laboratory, IMSUT.

To gain insights into signaling mechanisms underlying a variety of physiological and pathophysiological events, including NMJ formation, muscle atrophy, inflammation, tumorigenesis, and tumor metastasis, we have performed proteomic and transcriptomic analyses. We are investigating the roles of candidate proteins and genes that appear to be involved in each of these biological events. For instance, we recently performed transcriptomic analyses related to mucosal inflammation, which suggested the importance of Th-17-related pathways. Thus, we are investigating how the pathways play roles in mucosal inflammation by using a mouse model of human colitis. In addition, we have prepared experimental settings for other omic approaches such as metabolomic analysis.

9. Screening of chemical compound and siRNA libraries.

Eguchi, T., Tsoumpra, M., Inoue-Yamauchi, A., Ueta, R., Nagatoishi, S., Tsumoto, K., and Yamanashi, Y.

In addition to the omic analyses described above, we performed high throughput screenings of chemical compound and siRNA libraries, aiming to intervene in pathogenic signals or to gain insights into signaling mechanisms underlying a variety of biological events. We are investigating in vivo and in vitro effects, including therapeutic ones in mouse models of

human diseases, of hit compounds or down-regulation of candidate genes, and continue the ongoing screenings to further collect appropriate hit compounds and candidate genes that may regulate important signalings. We are also investigating target proteins for the hit compounds to understand their modes of actions.

Publications

Eguchi T., Yamanashi Y. Adeno-associated virus-mediated expression of an inactive CaMKIIβ mutant enhances muscle mass and strength in mice. Biochem Biophys Res Commun. 589: 192-196, 2021.

Department of Cancer Biology

Division of Cancer Cell Biology
癌防御シグナル分野

Professor	Makoto Nakanishi, M.D., Ph.D.	教　授	医学博士	中　西　　真
Associate Professor	Atsuya Nishiyama, Ph.D.	准教授	博士(理学)	西　山　敦　哉
Assistant Professor	Yoshikazu Johmura, Ph.D.	助　教	博士(薬学)	城　村　由　和
Assistant Professor	Sae Aratani, M.D., Ph.D.	助　教	博士(医学)	荒　谷　紗　絵

Several lines of evidence have underpinned the prominent role of senescent cells in aging and healthspan although a general understanding of molecular basis underlying senescence-associated pathogenesis is still in its early stages. Our research interests are to understand the common pathological basis underlying various age-associated disorders. We are currently focusing on in vivo dynamics of p16-positive senescent cells. To address this issue, we have analyzed in vivo p16-positive cells at a single cell level using a mouse model in which p16-positive senescent cells are visualized by fluorescent labelling. In addition, we are interested in elucidating the mechanisms underlying age-dependent accumulation of senescent cells in vivo. A molecular link between DNA methylation and genomic integrity is also under investigation.

1. A novel senolytic strategy targeting glutaminolysis ameliorates various age-associated disorders

Yoshikazu Johmura, Sae Aratani Chieko Konishi, Sayaka Yamane, Yoshie Chiba, Dan Li, Wang Tehwei, Takehiro Yamanaka, Harris Alexander Stephen, Narumi Suzuki, Satotaka Ohmori, Liu Xianuyang, Yue Zhang, Sakura Yumoto, Lindo Nakamura, Yao Jiayi, Kim Jiwoo, Toshiro Migita, Makoto Suematsu[1], Masaki Matsumoto[2], Makoto Arita[3], Masataka Sugimoto[4], Keiichi I Nakayama[5], Yoichi Furukawa[6], Seiya Imoto[7], and Makoto Nakanishi:

[1]Department of Biochemistry, Keio University School of Medicine,

[2]Department of Omics and Systems Biology, Niigata University Graduate School of Medical and Dental Sciences,

[3]RIKEN Center for Integrative Medical Sciences,

[4]Research Institute, National Center for Geriatrics and Gerontology,

[5]Department of Molecular and Cellular Biology, Medical Institute of Bioregulation, Kyushu University,

[6]Division of Health Medical Intelligence, Human Genome Center, [7]Division of Clinical Genome Research, IMSUT

Removal of senescent cells (senolysis) has been proposed to be beneficial for improving age-associated pathologies, but the molecular pathways for such senolytic activity have not yet emerged. In order to address this question, we performed genome wide screening to identify genes essential for senescence survival. We then identified glutaminase 1 (GLS1) as an essential gene for the survival of senescent cells. The intracellular pH in senescent cells was lowered by lysosomal membrane damage, and this lowered pH induced kidney-type glutaminase (KGA) expression. The resulting enhanced glutaminolysis induced the ammonia production, which neutralized the lower pH and improved survival of the senescent cells. Inhibition of KGA dependent glutaminolysis in aged mice eliminated senescent cells specifically and ameliorated age-associated organ dysfunction. Our re-

sults suggest that senescent cells rely on glutaminolysis, and its inhibition offers a promising strategy for inducing senolysis in vivo.

2. p53-Fbxo22-TFEB controls basal autophagy to govern hormesis

Narumi Suzuki, Yoshikazu Johmura, Chieko Konishi, Sayaka Yamane, Yoshie Chiba, Dan Li, Wang Tehwei, Takehiro Yamanaka, Harris Alexander Stephen, Satotaka Ohmori, Liu Xianuyang, Yue Zhang, Sakura Yumoto, Lindo Nakamura, Yao Jiayi, Kim Jiwoo, Toshiro Migita, Tamotsu Yoshimori[1], Tomohiko Ohta[2], and Makoto Nakanishi:
[1]Department of Genetics, Graduate School of Medicine, Osaka University
[2]Department of Translational Oncology, St. Marianna University Graduate School of Medicine

Preconditioning with a mild stressor such as fasting is a promising way to reduce severe side effects from subsequent chemo- or radio-therapy. However, the underlying mechanisms have been largely unexplored. In order to address this question, we examined the role of p53 in hermetic responses to a mild-stressor. We found that the p53-Fbxo22-TFEB axis played an essential role in this process through upregulating basal autophagy. Mild stress-activated p53 transcriptionally induced Fbxo22, which in turn ubiquitylated KDM4B complexed with Myc-N-CoR suppressors for degradation, leading to transcriptional induction of TFEB. Upregulation of autophagy related genes by increased TFEB dramatically enhanced autophagic activity and cell survival upon following severe stressor. Mitogen-induced AKT activation counteracted this process through phosphorylation of KDM4B, which inhibited Fbxo22-mediated ubiquitylation. Fbxo22$^{-/-}$ mice died within 10 hours of birth and their MEFs showed a lowered basal autophagy, whereas Fbxo22 overexpressing mice were resistant to chemotherapy. Taken together, these results suggest that p53 upregulates basal autophagy through the Fbxo22-TFEB axis, which governs the hormetic effect in chemotherapy.

3. Escape from senescence surveillance by PD-L1-PD1 immune checkpoint and its blockade ameliorates various senescence-associated disorders

Teh-Wei Wang, Yoshikazu Johmura, Narumi Suzuki, Satotaka Omori, Toshiro Migita, Kiyoshi Yamaguchi[1], Seira Hatakeyama[1], Satoshi Yamazaki[2], Eigo Shimizu[3], Seiya Imoto[3], Yoichi Furukawa[1], and Makoto Nakanishi
[1] Division of Clinical Genome Research, IMSUT
[2] Division of Stem Cell Research, IMSUT and Laboratory of Stem Cell Therapy, Faculty of Medicine, University of Tsukuba,
[3]Division of Health Medical Intelligence, Human Genome Center, IMSUT

Several lines of evidence have suggested that senescent cells accumulate in various tissues with age, which causes excess inflammation and a consequent imbalance in tissue homeostasis. A simple explanation for this accumulation might be the age-dependent increase in senescence-inducing stimuli such as telomere shortening and DNA damage. An impaired immune system might also be involved because oncogene-induced senescent hepatocytes and injury-induced senescent stellate cells have been recently reported to be eliminated by activated T cells and natural killer cells, respectively. However, precisely how the immune system surveils cellular senescence during natural aging is largely unknown.

we demonstrated that senescent cells expressed immune checkpoint PD-L1 in a heterogeneous manner. PD-L1^{+} senescent cells selectively accumulated with age *in vivo*. The heterogeneous expression of PD-L1 was at least in part due to impaired proteasomal activity in individual senescent cells. Although PD-L1^{-} cells were sensitive to T cell surveillance, PD-L1^{+} cells were resistant even in the presence of SASP. Intriguingly, single-cell analyses of *in vivo* 16^{high} cells revealed that PD-L1 expression was correlated with a high level of senescence-associated secretory phenotypes. Consistent with this, administration of monoclonal anti-PD-1 antibody to naturally aged and NASH-induced mice reduced the total number as well as the PD-L1^{+} population of p16^{high} cells *in vivo* and ameliorated various age- and senescence-associated disorders. Taken together, heterogeneous expression of PD-L1 appeared to play a key role in the age-dependent accumulation of senescent cells and inflammation. Specific elimination of PD-L1^{+} senescent cells by immune checkpoint blockade might provide a promising strategy for senolytic therapies.

4. The termination of UHRF1-dependent PAF15 ubiquitin signaling is regulated by USP7 and ATAD5

Ryota Miyashita, Atsuya Nishiyama, Chieko Konishi, Yoshie Chiba, Soichiro Kumamoto, Tomomi Nagatani, Hiroko Kozuka-Hata[1], Masaaki Oyama[1], and Makoto Nakanishi:
[1] Medical Proteomics Laboratory, IMSUT

UHRF1-dependent ubiquitin signaling plays an integral role in the regulation of maintenance DNA methylation. UHRF1 catalyzes transient dual mono-ubiquitylation of PAF15 (PAF15Ub2), which regulates the localization and activation of DNMT1 at DNA methylation sites during DNA replication. Although the initiation of UHRF1-mediated PAF15 ubiquitin signaling has been relatively well characterized, mechanisms underlying its termination and

how they are coordinated with the completion of maintenance DNA methylation have not yet been clarified.

To address this issue, we set out to study the molecular mechanism of PAF15 chromatin unloading to understand how the termination of maintenance DNA methylation is regulated. Using a cell-free system derived from *Xenopus* egg extracts that recapitulate the processes of maintenance DNA methylation, we demonstrated that the unloading of PAF15Ub2 is regulated by two regulatory mechanisms, namely USP7-dependent deubiquitylation and unloading of PAF15 by ATPase family AAA domain-containing protein 5 (ATAD5). We also found that PAF15 unloading is tightly coordinated with the completion of maintenance DNA methylation and requires the release of UHRF1 from chromatin. Finally, co-depletion of USP7 and ATAD5 from egg extracts results in an elevated global DNA methylation. We propose that timely inactivation of PAF15 is critical for the faithful inheritance of DNA methylation patterns.

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Department of Cancer Biology

Division of Aging and Regeneration
老化再生生物学分野

Professor	Emi K. Nishimura, M.D., Ph.D.	教　授	博士(医学)	西　村　栄　美
Associate Professor	Daisuke Nanba, Ph.D.	准教授	博士(理学)	難　波　大　輔
Assistant Professor	Yasuaki Mohri, Ph.D.	助　教	博士(農学)	毛　利　泰　彰
Project Assistant Professor	Kyosuke Asakawa, Ph.D.	特任助教	博士(工学)	浅　川　杏　祐

Stem cell systems play fundamental roles in sustaining tissue turnover and homeostasis. Our goal is to understand the mechanisms of tissue homeostasis in mammals and to apply that knowledge to better understand the mechanisms underlying tissue/organ aging, cancer development and other relevant diseases associated with aging. We further aim to apply this knowledge to drug discovery, regenerative medicine and the prevention and treatment of age-associated diseases.

1. Distinct types of stem cell divisions determine organ regeneration and aging in hair follicles

Matsumura H, Liu N[1], Nanba D, Ichinose S[2], Takada A[1], Kurata S[3], Morinaga H, Mohri Y, De Arcangelis A[4], Ohno S[5], and Nishimura EK.; [1]Department of Stem Cell Biology, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan; [2]Research Center for Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan; [3]Beppu Garden-Hill Clinic, Kurata Clinic, Beppu City, Japan; [4]Institut de Génétique et de Biologie Moléculaire et Cellulaire, Department of Development and Stem Cells, CNRS UMR7104, Inserm U1258, Université de Strasbourg, Illkirch, France; [5]Department of Molecular Biology, Yokohama City University School of Medicine, Kanazawa, Yokohama, Japan.

Hair follicles, mammalian mini-organs that grow hair, miniaturize during aging, leading to hair thinning and loss. Here we report that hair follicle stem cells (HFSCs) lose their regenerative capabilities during aging owing to the adoption of an atypical cell division program. Cell fate tracing and cell division axis analyses revealed that while HFSCs in young mice undergo typical symmetric and asymmetric cell divisions to regenerate hair follicles, upon aging or stress, they adopt an atypical 'stress-responsive type of asymmetric cell division. This type of division is accompanied by the destabilization of hemidesmosomal protein COL17A1 and cell polarity protein aPKCλ and generates terminally differentiating epidermal cells instead of regenerating the hair follicle niche. With the repetition of these atypical divisions, HFSCs detach from the basal membrane causing their exhaustion, elimination and organ aging. The experimentally induced stabilization of COL17A1 rescued organ homeostasis through aPKCλ stabilization. These results demonstrate that distinct stem cell division programs may govern tissue and organ aging.

2. Obesity accelerates hair thinning by stem cell-centric converging mechanisms

Morinaga H, Mohri Y, Grachtchouk M[1], Asakawa K, Matsumura H, Oshima M[2], Takayama N[3], Kato T, Nishimori Y, Sorimachi Y[4], Takubo K[4], Suganami T[5], Iwama A[2], Iwakura Y[6], Dlugosz A.A[1], and Nishimura E.K.; [1]Department of Dermatology, Universi-

ty of Michigan Medical School, Ann Arbor, MI, USA; [2]Division of Stem Cell and Molecular Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan; [3]Department of Regenerative Medicine, Graduate School of Medicine, Chiba University, Chiba, Japan; [4]Department of Stem Cell Biology, Research Institute National Center for Global Health and Medicine, Tokyo, Japan; [5]Department of Molecular Medicine and Metabolism, Research Institute of Environmental Medicine, Nagoya University, Nagoya, Japan; [6]Centre for Animal Disease Models, Research Institute for Biomedical Sciences, Tokyo University of Science, Chiba, Japan.

Obesity is a worldwide epidemic that predisposes individuals to many age-associated diseases, but its exact effects on organ dysfunction are largely unknown. Hair follicles—mini-epithelial organs that grow hair—are miniaturized by ageing to cause hair loss through the depletion of hair follicle stem cells (HFSCs). Here we report that obesity-induced stress, such as that induced by a high-fat diet (HFD), targets HFSCs to accelerate hair thinning. Chronological gene expression analysis revealed that HFD feeding for four consecutive days in young mice directed activated HFSCs towards epidermal keratinization by generating excess reactive oxygen species, but did not reduce the pool of HFSCs. Integrative analysis using stem cell fate tracing, epigenetics and reverse genetics showed that further feeding with an HFD subsequently induced lipid droplets and NF-κB activation within HFSCs via autocrine and/or paracrine IL-1R signalling. These integrated factors converge on the marked inhibition of Sonic hedgehog (SHH) signal transduction in HFSCs, thereby further depleting lipid-laden HFSCs through their aberrant differentiation and inducing hair follicle miniaturization and eventual hair loss. Conversely, transgenic or pharmacological activation of SHH rescued HFD-induced hair loss. These data collectively demonstrate that stem cell inflammatory signals induced by obesity robustly represses organ regeneration signals to accelerate the miniaturization of mini-organs, and suggests the importance of daily prevention of organ dysfunction.

3. Dynamic stem cell selection safeguards the genomic integrity of the epidermis

Kato T, Liu N, Morinaga H, Asakawa K, Muraguchi T, Muroyama Y, Shimokawa M, Matsumura H, Nishimori Y, Tan LJ, Hayano M[1,2,3], Sinclair DA[1,4], Mohri Y, Nishimura EK. ; [1]Department of Genetics, Blavatnik Institute, Paul F. Glenn Center for Biology of Aging Research, Harvard Medical School, Boston, MA, USA; [2]Department of Neuropsychiatry, Keio University School of Medicine, Shinjuku-ku, Tokyo, Japan; [3]Faculty of Science and Technology, Keio University, Yokohama, Japan; [4]Laboratory for Ageing Research, Department of Pharmacology, School of Medical Sciences, The University of New South Wales, Sydney, New South Wales, Australia

Maintaining genomic integrity and stability is crucial for life; yet, no tissue-driven mechanism that robustly safeguards the epithelial genome has been discovered. Epidermal stem cells (EpiSCs) continuously replenish the stratified layers of keratinocytes that protect organisms against various environmental stresses. To study the dynamics of DNA-damaged cells in tissues,we devised an in vivo fate tracing system for EpiSCs with DNA double-strand breaks (DSBs) and demonstrated that those cells exit from their niches. The clearance of EpiSCs with DSBs is caused by selective differentiation and delamination through the DNA damage response (DDR)-p53-Notch/p21 axis, with the downregulation of ITGB1. Moreover, concomitant enhancement of symmetric cell divisions of surrounding stem cells indicates that the selective elimination of cells with DSBs is coupled with the augmented clonal expansion of intact stem cells. These data collectively demonstrate that tissue autonomy through the dynamic coupling of cell-autonomous and non-cell-autonomous mechanisms coordinately maintains the genomic quality of the epidermis.

4. Stem cell spreading dynamics intrinsically differentiate acral melanomas from nevi.

Eshiba S, Namiki T[1], Mohri Y, Aida T[2,3], Serizawa N[4], Shibata T[5], Morinaga H, Nanba D, Hiraoka Y[2,3], Tanaka K[2], Miura K[6], Tanaka M[7], Uhara H[8], Yokozeki H[1], Saida T[9], Nishimura EK; [1]Department of Dermatology, Tokyo Medical and Dental University Graduate School and Faculty of Medicine,Tokyo, Japan; [2]Department of Molecular Neuroscience, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan; [3]Laboratory of Genome Editing for Biomedical Research, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan; [4]Department of Stem Cell Biology, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan; [5]Medical Corporation Shibata Dermatology Clinic, Osaka, Japan; [6]Department of Pathology, Tokyo Medical and Dental University Graduate School and Faculty of Medicine, Tokyo, Japan; [7]Department of Dermatology, Tokyo Women's Medical University Medical Center East, Tokyo, Japan; [8]Department of Dermatology, Sapporo Medical University School of Medicine, Hokkaido, Japan; [9]Shinshu University, Saitama, Japan

Early differential diagnosis between malignant

and benign tumors and their underlying intrinsic differences are the most critical issues for life-threatening cancers. To study whether human acral melanomas, deadly cancers that occur on non-hair-bearing skin, have distinct origins that underlie their invasive capability, we develop fate-tracing technologies of melanocyte stem cells in sweat glands (glandular McSCs) and in melanoma models in mice and compare the cellular dynamics with human melanoma. Herein, we report that glan-dular McSCs self-renew to expand their migratory progeny in response to genotoxic stress and trauma to generate invasive melanomas in mice that mimic human acral melanomas. The analysis of melanocytic lesions in human volar skin reveals that genetically unstable McSCs expand in sweat glands and in the surrounding epidermis in melanomas but not in nevi. The detection of such cell spreading dynamics provides an innovative method for an early differential diagnosis of acral melanomas from nevi.

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Department of Basic Medical Sciences

Division of Neuronal Network
神経ネットワーク分野

Professor	Toshiya Manabe, M.D., Ph.D.		教　授	医学博士	真　鍋　俊　也
Assistant Professor	Shizuka Kobayashi, Ph.D.		助　教	博士(生命科学)	小　林　静　香
Assistant Professor	Takahiko Chimura, Ph.D.		助　教	博士(理学)	千　村　崇　彦

Our major research interest is the molecular mechanisms of higher brain functions in mammals such as emotion and memory. We are especially focusing on the roles of functional molecules localized in synapses, for instance, neurotransmitter receptors, signal transduction molecules and adhesion molecules, in neuronal information processing. We are examining receptor functions, synaptic transmission and plasticity, and their roles in the whole animal with electrophysiological, biochemical, molecular genetic and behavioral approaches. We are also trying to elucidate fundamental aspects of psychiatrical and neurological disorders using model animals.

1. Cooperation of LIM domain-binding 2 (LDB2) with EGR in the pathophysiology of schizophrenia

Kiyama, Y., Arima-Yoshida, F., Ohnishi, T.[1], Yoshikawa, T.[1] and Manabe, T.: [1]Laboratory for Molecular Psychiatry, RIKEN Center for Brain Science, Wako, Saitama, Japan

Genomic defects with large effect size can help elucidate unknown pathologic architecture of mental disorders. We previously reported on a patient with schizophrenia and a balanced translocation between chromosomes 4 and 13 and found that the breakpoint within chromosome 4 is located near the *LDB2* gene. We show here that *Ldb2* knockout (KO) mice displayed multiple deficits relevant to mental disorders. In particular, *Ldb2* KO mice exhibited deficits in the fear-conditioning paradigm. Analysis of the amygdala suggested that dysregulation of synaptic plasticity controlled by the immediate-early gene *ARC* is involved in the phenotypes. We show that LDB2 forms protein complexes with known transcription factors. Consistently, ChIP-seq analyses indicated that LDB2 binds to >10,000 genomic sites in human neurospheres. We found that many of those sites, including the promoter region of *ARC*, are occupied by EGR transcription factors. Our previous study showed an association of the *EGR* family genes with schizophrenia. Collectively, the findings suggest that dysregulation in the gene expression controlled by the LDB2-EGR axis underlies a pathogenesis of subset of mental disorders.

2. Gastrin-releasing peptide regulates fear learning under stressed conditions via activation of the amygdalostriatal transition area

Goto, F., Kiyama, Y., Ogawa, I., Okuno, H.[2], Bito, H.[3], Yoshida, N.[4] and Manabe, T.: [2]Department of Biochemistry and Molecular Biology, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan, [3]Department of Neurochemistry, Graduate School of Medicine, University of Tokyo, Tokyo, Japan, [4]Laboratory of Developmental Genetics, Center for Experimental Medicine and System Biology, Institute of Medical Science, University of Tokyo, Tokyo, Japan

The amygdala, a critical brain region responsible

for emotional behavior, is crucially involved in the regulation of the effects of stress on emotional behavior. In the mammalian forebrain, gastrin-releasing peptide (GRP), a 27-amino-acid mammalian neuropeptide, which is a homolog of the 14-amino-acid amidated amphibian peptide bombesin, is highly expressed in the amygdala. The levels of GRP are markedly increased in the amygdala after acute stress; therefore, it is known as a stress-activated modulator. To determine the role of GRP in emotional behavior under stress, we conducted some behavioral and biochemical experiments with GRP-knockout (KO) mice. GRP-KO mice exhibited a longer freezing response than wild-type (WT) littermates in both contextual and auditory fear-conditioning tests only when they were subjected to acute restraint stress 20 min before the conditioning. To identify the critical neural circuits associated with the regulation of emotional memory by GRP, we conducted Arc/Arg3.1-reporter mapping in the amygdala with an Arc-Venus reporter transgenic mouse line. In the amygdalostriatal transition area (AST) and the lateral side of the basolateral nuclei, fear conditioning after restraint stress increased neuronal activity significantly in WT mice, and GRP KO was found to negate this potentiation only in the AST. These results indicate that the GRP-activated neurons in the AST are likely to suppress excessive emotional expression through the regulation of downstream circuits related to fear learning following acute stress.

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Department of Basic Medical Sciences

Division of Cell Signaling and Molecular Medicine

分子シグナル制御分野

Professor	Mutsuhiro Takekawa, M.D., Ph.D.	教　授	博士(医学)	武　川　睦　寛
Assistant Professor	Yuji Kubota, Ph.D.	助　教	博士(理学)	久保田　裕　二
Assistant Professor	Takanori Nakamura, Ph.D.	助　教	博士(理学)	中　村　貴　紀

The aims of the ongoing research projects in our laboratory are to elucidate the regulatory mechanisms of intracellular signal transduction systems responsible for cell-fate decisions, such as MAP kinase cascades and Stress granules. Perturbation of these signaling systems is involved in a variety of life-threatening diseases, including cancer, autoimmune diseases, neurodegenerative disorders and type 2 diabetes. Our laboratory also aims to develop new diagnostic or therapeutic tools for currently intractable disorders in which these pathways are involved.

1. Identification of novel substrates of human mitogen-activated protein kinases and their roles in human cancer.

Seina Oe, Mariko Saito, Hitomi Seki, Yuji Kubota, and Mutsuhiro Takekawa

Sequential activation of protein kinases within MAPK cascades is an evolutionary-conserved mechanism of intracellular signaling in the eukaryotic world. In mammals, at least three distinct subfamilies of MAPKs are present, namely, ERK, JNK, and p38. While the classical ERK MAPK is mainly activated by mitogenic stimuli, two relatively newly identified MAPKs, p38 and JNK, are preferentially activated by various environmental stresses. Therefore, p38 and JNK MAPKs are collectively called stress-activated protein kinases (SAPKs). Each of these MAPK cascades can regulate several different and sometimes overlapping biological functions. In general, the ERK pathway mediates growth-promoting and anti-apoptotic signaling, while the p38 and JNK pathways play pivotal roles in cellular stress responses such as growth arrest and apoptosis. In addition, the p38 and JNK pathways are involved in inflammatory responses. Perturbation of these crucial signal transduction pathways is involved in the pathophysiology of various life-threatening diseases, including cancer, autoimmune diseases, and neurodegenerative disorders.

Since these MAPKs exert their biological effects through the phosphorylation of their specific substrate proteins, identification of which is essential for understanding of regulatory mechanisms of critical biological phenomena. By developing a unique screening strategy using yeast cells, we have isolated several new types of human MAPK substrate proteins from human cDNA libraries. These substrates include regulatory molecules for the expression of growth-promoting genes, and for cell cycle progression and centrosome integrity, and protein kinases and phosphatases that regulate cell proliferation and inflammation. We confirmed that these molecules were indeed directly phosphorylated by one (or more) of the human MAPKs *in vitro* and *in vivo* in response to mitogenic and/or stress stimuli. Thus, these molecules are bona fide substrates of MAPKs. The

physiological relevance and pathological implications of these novel substrate proteins are currently under investigation in our laboratory.

2. Role of stress granule assembly in regulation of cellular stress response.

Daisuke Yoshioka, Aoi Matsuda, Takanaori Nakamura, and Mutsuhiro Takekawa

In dealing with environmental stresses, human cells either activate defense mechanisms to survive or initiate apoptosis, depending on the level and type of stress. One of the major cellular defense mechanisms is the assembly of stress granules (SGs). SGs are cytoplasmic ribonucleoprotein foci that appear when eukaryotic cells are exposed to specific types of stress such as ER stress, heat shock, hypoxia or viral infection. The core components of SGs are large aggregates of stalled translation pre-initiation complexes that contain mRNA, 40S ribosomal subunits, translation initiation factors and several RNA-binding proteins (RBPs). In general, the assembly of SGs is triggered by stress-induced phosphorylation of eIF2α, and requires self-oligomerization of certain RBPs such as G3BP. In cells under various stresses, eIF2α is phosphorylated by several different stress-sensing kinases. Phosphorylation of eIF2α suppresses productive translation initiation by preventing formation of the eIF2-GTP-Met-tRNAi complex. Under the stress conditions, specific RBPs such as G3BP1/2, instead of the ternary complex, interact with an mRNA in the 43S complex, leading to the assembly of a translationally stalled 48S complex. Self-oligomerization of RBPs by liquid-liquid phase separation (LLPS) promotes the formation of discrete cytoplasmic foci termed SGs. In addition to the standard mechanism, SGs can be formed by other mechanisms. For instance, drugs or lipid mediators that target eIF4F, such as pateamine A, hippuristanol and 15d-PGJ2, inhibit translation initiation and thereby initiate SG assembly independently of eIF2α phosphorylation.

We have previously reported that when cells are exposed to SG-inducing stresses, the signaling adaptor protein RACK1 is sequestered into SGs, and this sequestration inhibits the SAPK pathways and subsequent apoptosis. Thus, formation of SGs serves as a cellular adaptive defense mechanism and protects cells from apoptosis under adverse conditions, by regulating mRNA translation as well as by sequestering signaling molecules. This year, we discovered novel SG-components including nucleotide-binding proteins, cytoskeletal proteins, and signaling molecules. By analyzing some of these SG-components, we elucidated the molecular mechanism as to how SG assembly can broadly suppress stress-induced apoptosis, and unraveled a novel role of SG formation in cellular stress responses and in tumor progression. Furthermore, we also identified a novel molecular mechanism that elicits SG assembly under a certain type of stress conditions.

4. Regulation of the stress-responsive p38 and JNK MAPKs under stress conditions

Saeko Kawataki, Yukari Shiozaki, Hisashi Moriizumi, Noriko Nishizumi-Tokai, Takanori Nakamura, Yuji Kubota, Mutsuhiro Takekawa

We have previously identified three GADD45 family proteins as activators of the MTK1 MAPKKK. Although the optimal stress stimuli for each gene are different, all GADD45 family genes are induced by various stress stimuli such as DNA-damaging reagents and cytokines. Expression of any of these GADD45 proteins in cells leads to the activation of MTK1 and its downstream p38 and JNK MAPKs. GADD45-mediated activation of SAPK pathways is important particularly in the late phase of cellular stress responses, because it requires transcriptional induction and protein synthesis of GADD45 prior to activation of MTK1. Thus, GADD45-mediated MTK1 activation provokes delayed and prolonged activation of SAPK signaling, which is particularly important for cell fate decisions, such as apoptotic cell death and inflammation, under stress conditions. This year, by establishing MTK1 or GADD45 deficient cells, we investigated the regulation and function of MTK, and uncovered novel roles of MTK1 in DNA-damage response, inflammation, and cell growth control. Furthermore, using molecular imaging techniques, we elucidated unique spatio-temporal regulation of SAPK signaling molecules under certain stress conditions, and identified its role in the regulation of stress-induced pro-inflammatory cytokine production, apoptotic cell death, and embryonic development.

5. Identification of novel genes whose expression is controlled by MAPK signaling pathways.

Ayaka Sakurai, Shiho Sameshima, Junichiro Nashimoto, Mariko Saito, Yusuke Takagi, Noriko Nishizumi-Tokai, Yuji Kubota, and MutsuhiroTakekawa

The initial cellular response to various environmental cues, such as growth factors, environmental stresses, and cytokines, is the transcriptional regulation of a set of genes that control a wide variety of biological functions. MAPK signaling pathways are known to play crucial roles in this process. Previous studies have shown that MAPKs directly phosphorylate and activate a bunch of transcription factors and regulators. For instance, the transcription factor ELK-1, which is a member of the ternary complex factor (TCF) subfamily, is a substrate of ERK. TCFs interact with a second transcription factor, serum response

factor (SRF), and these two transcription factors jointly bind and activate serum response elements (SREs) in the promoters of immediately early genes (IEGs). Moreover, upon stress stimulation, p38 and JNK MAPKs directly phosphorylate activating transcription factor 2 (ATF2). ATF2 binds either to CRE response elements as a homodimer, or to both AP-1 and CRE sequences as a heterodimer, in which ATF2 forms a complex with other members of the ATF family or with Jun/Fos family members, thereby inducing target gene expression.

We have comprehensively searched for human genes whose expression is transcriptionally regulated by the MAPK signaling pathways, and have succeeded in identifying dozens of such genes. Interestingly, these transcripts include not only protein-coding mRNAs but also various non-coding, functional RNAs. We confirmed that some of these transcripts were indeed expressed preferentially in cancer cells with hyper-ERK activity or in cells treated with certain types of stresses. The roles of these MAPK-dependent transcripts in the regulation of cell fate decisions are currently under investigation in our laboratory.

Publications

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吉岡大介，中村貴紀，武川睦寛
ストレス顆粒形成による生命機能制御と疾患
実験医学増刊　「相分離　メカニズムと疾患」
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Department of Basic Medical Sciences

Division of RNA and Gene Regulation
RNA 制御学分野

Professor	Toshifumi Inada, Ph.D.	教　授	博士(理学)	稲　田　利　文
Associate Professor	Yoshitaka Matsuo, Ph.D.	准教授	博士(理学)	松　尾　芳　隆
Assistant Professor	Toru Suzuki, Ph.D.	助　教	博士(理学)	鈴　木　　亨

Translation quality controls eliminate aberrant proteins to maintain protein homeostasis and normal cell function. Improving the accuracy of translation and preventing the production of abnormal proteins is a practical approach for suppressing a series of neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. We analyze the molecular mechanism of quality control mechanisms that suppress abnormal proteins and clarify drug discovery's molecular basis. We propose that the increase in translation accuracy and the enhancement of translation quality control mechanisms are possible strategies to prevent abnormal protein production and prolong healthy life expectancy.

1. Quality control for translation abnormalities Molecular mechanism of RQC and NGD

Ribosome-associated Quality Control (RQC) monitors aberrant translations and decomposes and removes abnormal proteins during synthesis. RQC plays an extremely important role as the very early stage of maintaining protein homeostasis. We have reported a molecular mechanism that recognizes and dissociates stagnant ribosomes during translation elongation, the early stage of RQC. In the last several years, we have reported that E3 ubiquitin ligase Hel2 and its mammalian homolog ZNF598 are required for RQC, and the novel RQT complex is involved in the dissociation of the ubiquitinated ribosomes to subunits. We and Hegde lab have reported that E3 ubiquitin ligase recognizes collided ribosomes (Disome/Trisome), and the specific structure of collided ribosomes. We reported that the ubiquitination of uS10 by Hel2 was reconstituted. Next, we identified an RQT complex that specifically dissociates ubiquitinated ribosomes into subunits and reconstituted the reaction in vitro. We also elucidated the structures of ANKZF1 and 60S ribosomes that cleave peptidyl-tRNA on 60S. The collision of ribosomes also induces NGD (No-Go Decay) quality controls in conjugation with RQC and triggers endonucleolytic cleavage of mRNA in the collided ribosome. We also reported two pathways of NGD, mRNA cleavage coupled to the dissociation of collided ribosome response in RQC and mRNA cleavage independent of RQC at the vicinity of the collided ribosomes.

NEMF (Rqc2 in yeast) interacts with 60S RNCs and recruits Ltn1/Listerin, which ubiquitinates peptidyl-tRNAs on dissociated 60S subunits. In the 60S subunit, the Rqc2 protein catalyses the C-terminal extension of stalled tRNA-bound peptides with alanine and threonine residues (CAT-tails) in a non-canonical mRNA-independent elongation reaction. CAT-tailing enables the degradation of substrates that lack a Ltn1p-accessible ubiquitination site by exposing a lysine residue that is normally sequestered in the ribosome exit tunnel. In the context of nascent chain degradation in budding yeast, CAT-tailing is a failsafe mechanism that expands the range of RQC-degradable substrates. However, the physiological function of CAT-tailing remains elusive. We recently found that Failure to Degrade CAT-Tailed Proteins Disrupts Neuronal Morphogenesis and Cell Survival. NEMF, a mammalian RQC2 homolog, modifies trans-

lation products of nonstop mRNAs, major erroneous mRNAs in mammals, with a C-terminal tail mainly composed of alanine with several other amino acids. Overproduction of nonstop mRNAs induces NC aggregation and caspase-3-dependent apoptosis and impairs neuronal morphogenesis, which are ameliorated by NEMF depletion. Moreover, we found that homopolymeric alanine tailing at least partially accounts for CAT-tail cytotoxicity. These findings explain the cytotoxicity of CAT-tailed NCs and demonstrate physiological significance of RQC on proper neuronal morphogenesis and cell survival. We also found that the nascent polypeptide in the 60S subunit determines the Rqc2-dependency of ribosomal quality control. We propose that poly-tryptophan sequences induce Rqc2-independent RQC, suggesting that CAT-tailing in the 60S subunit could be modulated by the polypeptide in the ribosome exit tunnel.

1.1. The ribosome collision sensor Hel2 functions as preventive quality control in the secretory pathway.

Yoshitaka Matsuo[1] and Toshifumi Inada[1,2] : [1]Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai 980-8578, Japan. [2]Division of RNA and Gene Regulation, Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

Ribosome collision because of translational stalling is recognized as a problematic event in translation by the E3 ubiquitin ligase Hel2, leading to non-canonical subunit dissociation followed by targeting of the faulty nascent peptides for degradation. Although Hel2-mediated quality control greatly contributes to maintenance of cellular protein homeostasis, its physiological role in dealing with endogenous substrates remains unclear. This study utilizes genome-wide analysis, based on selective ribosome profiling, to survey the endogenous substrates for Hel2. This survey reveals that Hel2 binds preferentially to the pre-engaged secretory ribosome-nascent chain complexes (RNCs), which translate upstream of targeting signals. Notably, Hel2 recruitment into secretory RNCs is elevated under signal recognition particle (SRP)-deficient conditions. Moreover, the mitochondrial defects caused by insufficient SRP are enhanced by hel2 deletion, along with mistargeting of secretory proteins into mitochondria. These findings provide insights into risk management in the secretory pathway that maintains cellular protein homeostasis.

1.2. Optimized protocol for tRNA identification in the ribosomal complexes from human cell lines.

Tsuyoshi Udagawa[1], Moeka Seki[1] and Toshifumi Inada[1,2] : [1]Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai, Miyagi 981-8567, Japan. [2]The institute of Medical Sciences, The University of Tokyo, Minato-ku, Tokyo 108-8639, Japan.

We described a protocol for tRNA identification in the 60S ribosome-nascent peptide complex co-purified with Nuclear Export Mediator Factor (NEMF), a responsible factor for C-terminal alanine and threonine tailing of the nascent peptide. Our protocol is based on regular reverse transcription followed by quantitative Polymerase chain reaction (PCR). Although this method cannot distinguish between amino acid-charged and uncharged and base-modified and unmodified tRNAs, it is a convenient way to estimate the relative level of tRNA species and thus can be useful for researchers.

2. Molecular mechanism of quality control NRD for deficient ribosomes

The ribosome is the central machinery for protein synthesis responsible for accurate codon recognition and highly efficient peptide bond formation. The ribosome interacts with various factors to perform essential functions for gene expression. Since abnormal ribosomes generated during the synthesis cause various expression abnormalities, cells have a quality control mechanism Nonfunctional Ribosomal RNA Decay (NRD) recognizes and eliminates functionally defective ribosomes. We recently analyzed the quality control of ribosomes deficient in function due to base substitution mutations conserved in all species, essential for accurate codon recognition in 18S rRNA, and ubiquitin at the K212 residue of ribosomal protein uS3. We identified E3 ubiquitin ligases that are essential and involved. It was also revealed that the ubiquitinated stagnant 80S ribosome was dissociated into each subunit by Slh1, and then the abnormal 40S was degraded.

3. The function of ribosome dynamic modification in stress response

Synthesis and modification of secretory proteins in the endoplasmic reticulum are essential for cells. Accumulation of abnormal proteins in the endoplasmic reticulum is harmful to cells and therefore responds by inducing the UPR pathway. In *Saccharomyces cerevisiae*, the membrane protein Ire1 activated by endoplasmic reticulum stress splices the precursor mRNA of the transcription factor Hac1, and Hac1 is synthesized to induce transcription of chaperones. In higher eukaryotes, PARK phosphorylates eIF2α and suppresses cell-wide translation initiation. In the process of elucidating the physiological function of ribosomal ubiquitination, we discovered a novel translational regulation in the endoplasmic reticulum stress response. We discovered a novel translational control mechanism during endoplasmic reticulum stress in *S.*

cerevisiae and clarified that ubiquitination of the ribosomal protein eS7 by E3 ubiquitin ligase Not4 is essential.

4. Discovery of new molecular mechanisms that determine mRNA stability

The genetic information encoded by mRNA is converted into protein by the ribosome. The ribosome decodes the genetic information using the three base sequences on the mRNA as one reading frame (codon), but multiple types of codons encode most amino acids. Each codon corresponding to the same amino acid is called a synonymous codon, and the intracellular abundance of the corresponding tRNA is biased. The codons that are often used are called optimal codons, and whether synonymous codons are optimal is quantified according to the abundance of the corresponding tRNA, and the higher the amount of tRNA, the higher the optimality. Therefore, it is known that when translating mRNA with many codons with optimal codons, the elongation rate is high, and many proteins are synthesized. Codon optimality plays a crucial role in gene expression because the translation elongation rate is closely linked to the regulation of expression level and the folding and targeting of the peptide chain to be synthesized. In recent years, Coller lab reported that the optimality of codons regulates the inherent stability of individual mRNAs. It has been established that mRNAs with more optimal codons are stable and mRNAs with less optimal codons are unstable. The higher the optimal codon, the faster the translation elongation rate, so the optimal codon determines the mRNA's half-life. We have elucidated the molecular basis by which Ccr4-Not senses the translation elongation rate and determines the degradation rate as an mRNA stability-determining mechanism, in collaboration with Beckmann lab and Coller lab. It is widely known that the Ccr4-Not complex involved in transcription/degradation and translational repression of mRNA binds to mRNA via RNA-binding protein, but we first used biochemical techniques to Ccr4. Buschauer in Beckmann lab has found that the Not5 subunit of the Ccr4-Not complex binds directly to the ribosome. Subsequently, a comprehensive analysis by selective ribosome profiling was performed to characterize the mRNA translated by the ribosome to which the Ccr4-Not complex specifically binds. The results showed that codon-level analysis showed that the affinity between the Ccr4-Not complex and the ribosome showed a very strong inverse correlation with codon optimality. We clarified the actual state of the mRNA degradation control mechanism by codon optimization and the significance of synonymous codons in genetic information that had been unknown for many years. Abnormal translation rate regulation during protein synthesis causes severe defects in protein function, leading to disruption of protein homeostasis. Proteostasis disruption causes a wide range of cellular dysfunctions such as defective protein accumulation, organelle damage, and disruption of signaling pathways. It is thought to cause neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease, and aging. These results are expected to serve as a basis for understanding the pathogenic mechanism and aging mechanism of diseases caused by the synthesis of function-deficient proteins due to translation abnormalities.

4.1. The ubiquitination-deubiquitination cycle on the ribosomal protein eS7A is crucial for efficient translation.

Yuka Takehara[1], Hideki Yashiroda[1], Yoshitaka Matsuo[2], Xian Zhao[1], Akane Kamigaki[1], Tetsuo Matsuzaki[1], Hidetaka Kosako[3], Toshifumi Inada[2,4], and Shigeo Murata[1]; [1]Laboratory of Protein Metabolism, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-0033, Japan. [2]Graduate School of Pharmaceutical Sciences, Tohoku University, Aoba-ku, Sendai 980-8578, Japan. [3]Division of Cell Signaling, Fujii Memorial Institute of Medical Sciences, Tokushima University, 3-18-15 Kuramoto-cho, Tokushima 770-8503, Japan. [4]Department of RNA and Gene Regulation, Institute of Medical Science, The University of Tokyo, Minato-ku, Tokyo 108-8639, Japan.

Ubiquitination is a major post-translational modification of ribosomal proteins. The role of ubiquitination in the regulation of ribosome functions is still being elucidated. We show that the cycle of ubiquitination and deubiquitination of the 40S ribosome subunit eS7 is important for efficient translation. eS7 ubiquitination at lysine 83 is required for efficient protein translation. We identified Otu2 and Ubp3 as the deubiquitinating enzymes for eS7. An otu2Dubp3D mutation caused a defect in protein synthesis. Ubp3 inhibited polyubiquitination of eS7 in polysomes to keep eS7 in a mono-ubiquitinated form, whereas Otu2 was specifically bound to the free 40S ribosome and promoted the dissociation of mRNAs from 40S ribosomes in the recycling step. Our results provide clues for understanding the molecular mechanism of the translation system via a ubiquitination-deubiquitination cycle.

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Human Genome Center

Laboratory of Functional Analysis *In Silico*
機能解析イン・シリコ分野

Professor	Kenta Nakai, Ph.D.	教　授　博士(理学)	中　井　謙　太
Associate Professor	Sung-Joon Park, Ph.D.	准教授　博士(工学)	朴　　聖　俊

Laboratory of Genome Database
ゲノムデータベース分野

Professor	Kenta Nakai, Ph.D.	教　授　博士(理学)	中　井　謙　太

The mission of our laboratory is to conduct computational ("in silico") studies on the functional aspects of genome information. At present, we mainly focus on the analysis of regulatory information of gene expression in the non-coding region, using a variety of next generation sequencing (NGS) data. In addition, we are actively collaborating with researchers from various fields.

1. Comprehensive comparison of gene expression diversity among human stem cells

Yukiyo Yamatani and Kenta Nakai

In stem cells *in vitro*, several factors including their tissue origins and culturing conditions affect their gene expression, sometimes resulting in changing their state or identity. However, the extent to which these factors affect the gene expression profile for each cell type remains unclear. Thus, we aimed to characterize four types of human stem cells from the effects of the various factors, integrating publicly available RNA-seq data. Induced pluripotent stem cells (iPSCs) were classified by their tissue origins. Besides, embryonic stem cells (ESCs) with different culture medium compositions were contained in the iPSC clusters. In mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs), culturing conditions mainly affected their expression profiles. We further investigated the genes identified stem cell clusters. Highly correlated factors in stem cell types were affected by expression changes of stem cell specific genes. Moreover, we observed high correlation between histone genes and zinc-finger genes in a subclass of cells characterized by their cell growth-related conditions, suggesting the existence of a common enhanced state across stem cell types. Overall, we suggest that the culturing conditions more strongly impact the stem cell identity than their types and tissue origins. Our findings would be helpful for controlling the stem cell quality.

2. Identification of novel gene expression networks that contribute to GBM genetic heterogeneity using scRNA-seq datasets

Taisuke Hori[1], Sung-Joon Park and Kenta Nakai

[1]Nippon Shinyaku, Co. ltd.

Glioblastoma multiforme (GBM) is known as the most common malignant brain tumor in adults. The main difficulties underlying drug development of this fatal tumor are due in part to its heterogeneity. In this study, to identify new therapeutic targets, we searched for gene expression programs that potentially associate with malignant properties of GBM by analyzing public scRNA-seq datasets. As a result, we identified a novel co-expressed gene module in single cell level which is the recurrent program within several donors. This module remarkably includes an amount of lncRNAs (>30%), as well as multiple tumor-related genes (referred to "NC module" hereafter). To understand the functional importance of the NC module, we applied the monocle algorithm that infers trajectories based on the similarity of gene expression patterns. As a result, we found that the cell populations with high expression of the NC module are concentrated at the trajectory termination. This result suggests that cells expressing NC module genes tend to be functionally stable and contribute to the genetic heterogeneity of GBM. Furthermore, we retrieved 14 hub genes from the NC module, which is the key component in its co-expressed gene network and plays an important role in controlling specific functional states of GBM. For example, ASTN2 (Astrotactin 2), a known gene regulating the migration of fetal neurons, is a hub gene and may contribute to the high degree of migration and invasion of GBM, to be a highly potential therapeutic target.

3. Constructing a data integration platform for the development of therapeutic agents of COVID-19

Sung-Joon Park, Katsunori Fujiki[2], Satoshi Otaki, Kenta Nakai, Yumiko Imai[3] and Katsuhiko Shirahige[2]
[2]Institute for Quantitative Biosciences, The University of Tokyo
[3]National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN)

In the medical treatment for patients with respiratory failure including new coronavirus infection COVID-19, there are still many unexplained factors that influence the effect of therapeutic drugs and vaccines. To contribute to the development of therapeutic drugs for COVID-19, by collecting bronchoalveolar lavage fluid samples of patients with COVID-19 and integrating the information of medical records, we are developing effective next-generation sequencing (NGS) protocols and the data analysis platform. With the time-course viral DNA and RNA sequencing data, we tuned our pipeline for meta-genome analysis and quantified microbe-originated NGS reads. We found severe bacterial and viral infections known to be involved, such as myocarditis, pericarditis, and pneumonia. Furthermore, the patients' severities correlated with the profile of detected microbes. Therefore, we believe that the information of bacterial and viral microorganisms significantly present in patients with COVID-19 is a useful resource to establish the therapeutic strategy.

4. Developing an open-access repository for the multi-dimensional genome structure data

Sung-Joon Park, Katsuhiko Shirahige[2], Shoji Takada[4] and Tomoko Nishiyama[5]
[4]Department of Biophysics, Graduate School of Science, Kyoto University
[5]Division of Biological Sciences, Graduate School of Science, Nagoya University

The community-wide effort to characterize 3D genome organization has highlighted the importance of functional linkages between genetic/epigenetic phenomena and physical properties of DNA (e.g., stiffness, torsion, and supercoiling). However, mechanisms underlying the establishment of functional genome structure are still poorly understood, which needs comprehensive and integrative approaches. Here, we are developing a data repository system that facilitates online in-silico analyses, named Genome Modality Suite as a part of the research project Genome Modality. The system has been designed to deal with heterogeneous and multi-dimensional data, such as RNA-seq and ChIP-seq signal tracks (1D data), Hi-C contact matrix (2D data), XYZ-coordinate structures (3D data). Furthermore, by utilizing PHP, MySQL, and JavaScript libraries, we successfully developed the prototype of a web-based browser to provide seamless access to the 123D data. Our system will accelerate progress toward the understanding of multi-dimensional genome properties.

5. Genome-wide in silico analysis for understanding the epigenetic mechanism in myeloid leukemogenesis

Sung-Joon Park, Shuhei Asada[6], Kenta Nakai, Susumu Goyama[7] and Toshio Kitamura[6]
[6]Division of Cellular Therapy
[7]Department of Computational Biology and Medical Science, Graduate School of Frontier Sciences, The University of Tokyo

In oncogenesis, the dysregulated epigenetic regulators involved in such as DNA methylation, histone modifications are recurrently detected. In particular, polycomb group proteins (PcGs)-mediated ubiquitination and de-ubiquitination are well-known epigenetic silencers of developmental genes, and the mutated PcGs lead to aberrant gene expression regulation. However, its mechanism is still largely unknown.

Here, we focused on the interplay of polycomb-repressive de-ubiquitinase (PR-DUB) and the dynamic change in H2AK119 mono-ubiquitination in mitotic myeloid leukemia cells. We performed in silico analyses with the next-generation sequencing data including ChIP-seq and ATAC-seq to profile the enrichments of ASXL1 (a component of PR-DUB), CDC20 (a coactivator of the E3 ubiquitin ligase complex APC/C), and H2AK119ub1 in 293T and NOMO-1 cells. We found the co-enrichment of ASXL1 with CDC20 in the promoter regions and that H2AK119ub1 was markedly reduced during mitosis. In contrast, the ChIP-seq data from the overexpression of CDC20 in myeloid leukemia cells exhibited markable H2AK119ub1 enrichment. With our in vitro and in vivo experimental results that the ectopic expression of CDC20 leads to an attenuation of myeloid leukemogenesis, the results suggest that APC/C induces the accumulation of H2AK119ub1 and acts as a tumor suppressor by counteracting the PR-DUB complex in myeloid leukemia.

6. Integrative transcriptomic analysis for lacrimal gland organoids generated from human pluripotent stem cells

Sung-Joon Park, Toru Okubo[8], Yuki Ishikawa[8] and Ryuhei Hayashi[8]
[8]Department of Stem Cells and Applied Medicine, Osaka University Graduate School of Medicine

Lacrimal glands that maintain a healthy tear-film are a target tissue for regenerative therapy and drug discovery using human pluripotent stem cells, where its dysfunction occurs in one of the most common autoimmune diseases, Sjögren's syndrome. In this study, using a SEAM (Self-formed, Ectodermal, Autonomous, Multi-zone) that is a 2D eye-like organoid generated from the 201B7 human iPS cells, lacrimal gland-like primordia were successfully sorted and subjected to the 3D culture that showed budding, with a multi-branched gland-like morphology emerging at day 14. To investigate molecular processes during the differentiation, we performed time-course transcriptomic analyses incorporating microarray, bulk RNA-seq, and single-cell RNA-seq data. These analyses revealed that transcription factors involved in lacrimal gland development (e.g., PAX6, SOX9, BARX2, RUNX1, SIX2, and FGFR2) were transiently upregulated during lacrimal gland differentiation culture. Single-cell RNA-seq indicated that at day 10 of 3D cultivation, lacrimal gland-like progenitors transiently expressed the proliferation marker, MKI67, along with epithelial-mesenchymal transition markers, VIM and FN1. The expression of these markers decreased by day 20, and AQP5-positive acinar cells started to express the functional lacrimal gland proteins, LCN28 and DEFB1, for lipocalin and defensin, respectively. The day-20 organoids, moreover, contained major cell clusters including acinar, ductal, and myoepithelial-like cells. These expression phenotypes during the cultivation suggest that lacrimal gland maturation starts during in vitro 3D culture and will continue in situ after implantation, which is an advantage for use in future regenerative therapies to treat disorders. Indeed, these immature organoids transplanted above the rat eyes properly functioned with an increased expression of lacrimal gland-related differentiation markers.

7. Investigating putative dendritic cell precursors (pre-DC) with neutrophil progenitor properties

Phit Ling Tan, Ken Ishii[9], Florent Ginhoux[10] and Kenta Nakai
[9]Division of Vaccine Science
[10]Singapore Immunology Network (SIgN), A*STAR

Previously, we found a potentially interesting new group of cells while studying the aggregated single-cell transcriptome data of human blood/bone marrow cells: a subtype of putative dendritic cell precursors (pre-DCs) that expresses certain neutrophil progenitor markers. Other than expressing these markers, this subtype exhibits similar expression profiles to conventional dendritic cells (cDCs) and $Axl^+Siglec6^+$pre-cDCs. Trajectory inference analyses suggest that this group is a precursor of DCs. After further investigations, we found that these cells are divided into two sub-groups: one is $Axl^+Siglec6^+$ pre-cDCs, whereas the other is located among $CD33^+$ granulocyte-monocyte progenitors (GMPs). GMPs are commonly known to be homogeneous oligopotent cells. However, recent studies have proposed that GMPs are heterogeneous. Furthermore, a group of early uni-potent neutrophil progenitors was revealed in GMPs. This suggests the existence of early uni-potent DC progenitors in GMPs. To verify the hypothesis, identification of cell surface markers of the putative pre-DCs are necessary. COMET, a tool for selection of candidate marker panels from scRNA-seq data, suggests LGALS1+ITGAX and LGALS1+HAVCR2 as the potential marker panels.

8. scIMC: a customized platform for benchmarking comparison and visualization analysis of imputation methods for single-cell RNA sequencing data

Chichi Dai[11], Ran Su[11], Kenta Nakai and Leyi Wei[12]
[11]College of Intelligence and Computing, Tianjin University
[12]School of Software, Shandong University

With the advent of scRNA-seq data, one major challenging is the so-called 'dropout events' that distort gene expression and remarkably influence downstream analysis in single-cell transcriptome. To ad-

dress this issue, much effort has been done and developed several scRNA-seq imputation methods, including mode-based and deep learning-based methods. However, comprehensively and systematically compare existing methods are still lacking. In this work, we use 2 real scRNA-seq datasets and 6 simulated datasets to comprehensively evaluate and compare the ability of a total of 11 available imputation methods from the following aspects: (1) evaluation of recovering true gene expression distribution, (2) cell clustering analysis, (3) gene differential expression analysis, and (4) evaluation of reconstructing cellular trajectories. Based on comparative results, we observe that no one method can outperform other methods in all respects. In addition, we present the first online platform, namely *scIMC*, that integrates all available state-of-the-art imputation methods for comparative analysis and provide visualization results.

9. PepBCL: An interpretable contrastive learning framework based on protein language model to predict protein-peptide binding residues using protein sequences only

Ruheng Wang[12], Junru Jin[12], Kenta Nakai and Leyi Wei[12]

Identifying the protein-peptide binding residues is fundamentally important to understand the mechanisms of protein functions and drug discovery. Although several computational methods have been developed, they highly rely on third-party tools or information for feature design, easily resulting in low computational efficacy and suffering from low predictive performance. To address the limitations, we propose PepBCL, a novel BERT (Bidirectional Encoder Representation from Transformers)-based Contrastive Learning framework to predict the protein-Peptide binding residues based on protein sequences only. PepBCL is an end-to-end predictive model that is free with feature design. We demonstrate that our proposed method significantly outperforms the state-of-the-art methods under benchmarking comparison and achieves more robust performance. Moreover, we found that we further improve the performance via the integration of traditional features and our learnt features. Our results highlight the flexibility and adaptability of deep learning-based protein language model to capture both conserved and non-conserved sequential characteristics of peptide-binding residues.

10. Spatial transcriptome analysis of the brain of *Ciona intestinalis*

Takehiro Kusakabe[13], Xin Zeng, Yutaka Suzuki[7] and Kenta Nakai
[13]Faculty of Science and Engineering, Konan University

As one of the closest living relatives of vertebrates, the ascidian *Ciona intestinalis* serves a critical role in understanding developmental and physiological processes that are comparable to – but far less complex than – those of vertebrates. The central nervous system (CNS) of *Ciona* consists of approximately 330 cells, of which around 177 are thought to be neurons. We aim to integrate scRNA-seq data of *Ciona* with spatial transcriptomics to analyze its brain function. To generate spatial maps of gene expression in the Ciona brain of its larvae and adult stages, *Ciona* samples are being prepared with the recently released 10x genomics Visium spatial transcriptomics system.

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Human Genome Center

Laboratory of Molecular Medicine
ゲノム医科学分野

Professor	Tatsuhiro Shibata, M.D., Ph.D.	教　授	医学博士	柴　田　龍　弘
Senior Assistant Professor	Atsushi Niida, Ph.D.	講　師	博士(理学)	新井田　厚　司
Assistant Professor	Takahashi Kazuki, Ph.D.	助　教	博士(農学)	髙　橋　数　冴

The Laboratory of Molecular Medicine focuses on comprehensive characterization of currently-untreatable diseases including cancer on the basis of molecular genomics and aims to make "breakthroughs for human health" by identifying novel disease-related genes/pathways, including potential therapeutic or preventive targets and biomarkers, and to understand human diseases as heterogeneous but intervention-able "biological systems". This group has also organized the facility for the analysis of next-generation high-performance sequencers

1. Mutational signatures in esophageal squamous cell carcinoma from eight countries with varying incidence

Esophageal squamous cell carcinoma (ESCC) shows remarkable variation in incidence that is not fully explained by known lifestyle and environmental risk factors. It has been speculated that an unknown exogenous exposure(s) could be responsible. Here we combine the fields of mutational signature analysis with cancer epidemiology to study 552 ESCC genomes from eight countries with varying incidence rates. Mutational profiles were similar across all countries studied. Associations between specific mutational signatures and ESCC risk factors were identified for tobacco, alcohol, opium and germline variants, with modest impacts on mutation burden. We find no evidence of a mutational signature indicative of an exogenous exposure capable of explaining differences in ESCC incidence. Apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like (APOBEC)-associated mutational signatures single-base substitution (SBS)2 and SBS13 were present in 88% and 91% of cases, respectively, and accounted for 25% of the mutation burden on average, indicating that APOBEC activation is a crucial step in ESCC tumor development.

2. Comprehensive Genomic Profiling of Neuroendocrine Carcinomas of the Gastrointestinal System

The neuroendocrine carcinoma of the gastrointestinal system (GIS-NEC) is a rare but highly malignant neoplasm. We analyzed 115 cases using whole-genome/exome sequencing, transcriptome sequencing, DNA methylation assays, and/or ATAC-seq and found GIS-NECs to be genetically distinct from neuroendocrine tumors (GIS-NETs) in the same location. Clear genomic differences were also evident between pancreatic NECs (Panc-NECs) and non-pancreatic GIS-NECs (Nonpanc-NECs). Panc-NECs could be classified into two subgroups (i.e., 'Ductal-type' and 'Acinar-type') based on genomic features. Alterations in TP53 and RB1 proved common in GIS-NECs and most Non-panc-NECs with intact Rb demonstrated mutually exclusive amplification of CCNE1 or MYC. Alterations of the Notch gene family were characteristic of Non-panc-NECs. Transcription factors for neuroendocrine differentiation, especially the SOX2 gene, appeared overexpressed in most GIS-NECs due to hypermethylation of the promoter region. This first comprehensive study of genomic alterations in GIS-NECs uncovered several key biological processes underlying genesis of this very lethal form of cancer.

3. The Evolving Genomic Landscape of Esophageal Squamous Cell Carcinoma Under Chemoradiotherapy

Esophageal squamous cell carcinoma (ESCC) often recurs after chemoradiotherapy, and the prognosis of ESCC after chemoradiotherapy has not improved over the past few decades. The mutation process in chemoradiotherapy-resistant clones and the functional relevance of genetic alterations remain unclear. To address these problems, we performed whole-exome sequencing of 52 tumor samples from 33 patients with ESCC who received radiotherapy combined with 5-fluorouracil/platinum. In multi-region analyses of pretreatment and locally recurrent lesions from five cases, most driver gene-altered clones remained under chemoradiotherapy selection pressure, while few driver gene alterations were acquired at recurrence. The mutation signatures of recurrent ESCC, including increased deletion frequency and platinum dose-dependent base substitution signatures, were substantially different from those of primary ESCC and reflected the iatrogenic impacts of chemoradiotherapy. Single-region analysis of 28 pretreatment tumors indicated that focal copy-number gain at the MYC locus was significantly associated with poor progression-free survival and overall survival after chemoradiotherapy. MYC gain remained throughout the chemoradiotherapy course and potentially contributes to intrinsic resistance to chemoradiotherapy. Consistent with these findings, MYC copy number and mRNA and protein levels in ESCC cell lines correlated positively with resistance to radiotherapy, and MYC knockdown improved sensitivity to radiotherapy. Overall, these data characterize the clonal evolution process induced by chemoradiotherapy and clinically relevant associations for genetic alterations in ESCC. These findings increase our understanding of therapeutic resistance and support the rationale for precision chemoradiotherapy.

4. Analysis of large-scale genomic data to elucidate the cancer evolution

Cancer is an abnormally proliferating cell that arise from the accumulation of genome mutation in healthy somatic cells. The process by which a somatic cell acquires and accumulates multiple genomic mutations to become a cancer cell can be regarded as the 'evolution' of the somatic cell. Elucidating the process of evolution into cancer cells leads to the elucidation of the mechanism of carcinogenesis and the intra tumor heterogeneity involved in the acquisition of drug resistance in cancer. It has been shown that the evolutionary status of cancer varies among cancer types, among cancer patients, and even among different stages of cancer progression. However, the factors that cause these changes of evolutionary state are completely unknown. We accumulated and analyzed the recently published large-scale whole genome sequencing data and several multi-region sampling data from various studies which conducted to elucidate the composition of cancer. In addition, we simulated the cancer cells evolution to elucidate the genomic variation and environmental factors involved in the changes of cancer evolution.

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Laboratory of Genome Technology
シークエンス技術開発分野

Professor	Tatsuhiro Shibata, M.D., Ph.D.	教　授	医学博士	柴　田　龍　弘
Professor	Koichi Matsuda, M.D., Ph.D.	連携教授（新領域創成科学研究科）	博士（医学）	松　田　浩　一
Assistant Professor	Chizu Tanikawa, Ph.D.	助　教	博士（医学）	谷　川　千　津

The major goal of our group is to identify genes of medical importance, and to develop new diagnostic and therapeutic tools. We have been attempting to isolate genes involving in carcinogenesis and also those causing or predisposing to various diseases as well as those related to drug efficacies and adverse reactions. By means of technologies developed through the genome project including a high-resolution SNP map, a large-scale DNA sequencing, and the cDNA microarray method, we have isolated a number of biologically and/or medically important genes, and are developing novel diagnostic and therapeutic tools.

1. A cross-population atlas of genetic associations for 220 human phenotype.

Current genome-wide association studies do not yet capture sufficient diversity in populations and scope of phenotypes. To expand an atlas of genetic associations in non-European populations, we conducted 220 deep-phenotype genome-wide association studies (diseases, biomarkers and medication usage) in BioBank Japan (n = 179,000), by incorporating past medical history and text-mining of electronic medical records. Meta-analyses with the UK Biobank and FinnGen (ntotal = 628,000) identified ~5,000 new loci, which improved the resolution of the genomic map of human traits. This atlas elucidated the landscape of pleiotropy as represented by the major histocompatibility complex locus, where we conducted HLA fine-mapping. Finally, we performed statistical decomposition of matrices of phenome-wide summary statistics, and identified latent genetic components, which pinpointed responsible variants and biological mechanisms underlying current disease classifications across populations. The decomposed components enabled genetically informed subtyping of similar diseases (for example, allergic diseases). Our study suggests a potential avenue for hypothesis-free re-investigation of human diseases through genetics.

A single nucleotide polymorphism in Prostate Stem Cell Antigen is associated with endoscopic grading in Kyoto classification of gastritis

The risk allele of a single nucleotide polymorphism (SNP) rs2294008 in the Prostate stem cell antigen (PSCA) gene is strongly associated with gastric cancer. Although the Kyoto classification score is believed to be an indicator of gastric cancer risk, it lacks supporting genetic evidence. We investigated the effect of this risk allele of PSCA SNP on the Kyoto score. Participants without a history of gastric cancer or Helicobacter pylori (H. pylori) eradication underwent esophagogastroduodenoscopy, H. pylori evaluation, and SNP genotyping. The Kyoto score is the sum of scores obtained from endoscopy-based atrophy, intestinal metaplasia, enlarged folds, nodularity, and diffuse redness. The Kyoto score is novel in the light of scoring for gastritis. A total of 323 patients were enrolled (number of individuals with genotype CC: 52; CT: 140; TT: 131, average age: 50.1 years, male: 50.8%). The patient baseline characteristics including age, sex, body mass index, smoking, drinking, family

history of gastric cancer, and H. pylori status had no association with PSCA SNP. The Kyoto score was higher in T (CT or TT genotype; risk allele) carriers than in CC carriers. Atrophy, enlarged folds, and diffuse redness scores were higher in T allele carriers (risk allele) than in CC genotype individuals. In multivariate analysis, the Kyoto score was independently associated with PSCA SNP (OR: 1.30, p = 0.012). Thus, the Kyoto score was associated with a genetic predisposition.

Combined landscape of single-nucleotide variants and copy number alterations in clonal hematopoiesis

Clonal hematopoiesis (CH) in apparently healthy individuals is implicated in the development of hematological malignancies (HM) and cardiovascular diseases. Previous studies of CH analyzed either single-nucleotide variants and indels (SNVs/indels) or copy number alterations (CNAs), but not both. Here, using a combination of targeted sequencing of 23 CH-related genes and array-based CNA detection of blood-derived DNA, we have delineated the landscape of CH-related SNVs/indels and CNAs in 11,234 individuals without HM from the BioBank Japan cohort, including 672 individuals with subsequent HM development, and studied the effects of these somatic alterations on mortality from HM and cardiovascular disease, as well as on hematological and cardiovascular phenotypes. The total number of both types of CH-related lesions and their clone size positively correlated with blood count abnormalities and mortality from HM. CH-related SNVs/indels and CNAs exhibited statistically significant co-occurrence in the same individuals. In particular, co-occurrence of SNVs/indels and CNAs affecting DNMT3A, TET2, JAK2 and TP53 resulted in biallelic alterations of these genes and was associated with higher HM mortality. Co-occurrence of SNVs/indels and CNAs also modulated risks for cardiovascular mortality. These findings highlight the importance of detecting both SNVs/indels and CNAs in the evaluation of CH

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Division of Health Medical Intelligence
健康医療インテリジェンス分野

Professor	Seiya Imoto, Ph.D.		教　授	博士(数理学)	井　元　清　哉
Project Associate Professor	Yao-zhong Zhang, Ph.D.		特任准教授	博士(情報理工学)	張　　耀　中

Laboratory of Sequence Analysis
シークエンスデータ情報処理分野

Professor	Seiya Imoto, Ph.D.	教　授	博士(数理学)	井　元　清　哉
Associate Professor	Kotoe Katayama, Ph.D.	准教授	博士(情報学)	片　山　琴　絵

Our mission is to realize genomic medicine based on the integrated data analysis of whole genomes of human and commensal microbiota by supercomputing. Development of computational data analysis methods including artificial intelligence for genomic, health, and medical big data is one of our main focuses. We promote integrative analysis of human whole genome, RNA and other omics data, commensal microbiota including bacteriome and virome, and health and medical-related big data. Furthermore, health medical intelligence aims at using the analysis results of such big data to create personalized health-medical action plan of individuals.

1. Whole Genome Sequencing and Genomic Medicine

a. Creating New Genomic Medicine by Integrating Human Whole Genome and Commensal Microbiota

Katayama K, Shimizu E, Kasajima R, Yamaguchi K, Yokoyama K, Yadome M, Hyugaji T, Komura M, Yamamoto M, Saito A, Fujimoto K, Kobayashi M, Ogawa M, Takei T, Yasui H, Yuji K, Takane K, Ikenoue T, Robert B, Shibuya T, Hiroshima Y, Hasegawa T, Miyagi Y, Muto K, Goyama S, Shida D, Boku N, Takahashi S, Nanya Y, Furukawa Y, Miyano S, Yamaguchi R, Uematsu S, Imoto S

Using state-of-the-art genome analysis and artificial intelligence, our mission is to implement “new genomic medicine” by integrating human genome information and human symbiotic microbial metagenome information.

In Japan, gene panel testing was covered by national health insurance from Jun 2019, however, it analyzed several hundreds of genes, which were known cancer-related genes. Since the gene panel has trivial limitation due to its focused genes, Japanese government considered to extend the gene panel to whole genome. However, it remains a question that whether the whole genome sequence information is enough to realize precision medicine.

Although human genome has 20 thousand genes, intestinal microbiota has 20 million genes, and they

work together with human genes for keeping homeostasis of our lives. In recent years, with the advancement of sequencing technology, we could have a whole figure of intestinal microbiota and found its dysbiosis leads to various diseases. We are proceeding a research for utilizing the information of intestinal microbiota (meta-genome) and human genome to create new genomic medicine in Society5.0. For this purpose, we need to establish an artificial intelligence to translate the information of human genome and meta-genome to clinical actions of physicians.

b. Establishment of Data Analysis Center in Implementation Plan for Whole Genome Analysis of Ministry of Health, Labour and Welfare

Katayama K, Shibuya T, Yamaguchi R, Mano H[1], Shiraishi Y[1], Sudo T[1], Oogaito T[1], Tamai I[1], Kohno T[1], Kato M[1], Oe K[2], Kataoka K[1], Okada Y[3], Fujimoto A[2], Imoto S: [1]National Cancer Center, Japan, [2]University of Tokyo School of Medicine, Japan, [3]Osaka University, Japan

Based on the Whole Genome Analysis Action Plan (Version 1) formulated on December 20, 2019 by the Ministry of Health, Labour and Welfare, the AMED project was launched in 2021 aiming at returning the result of WGS analysis to the patients as medical actions. This national project covers a wide range of intractable cancers, including gastrointestinal, hematological, pediatric, rare, gynecological, and respiratory cancers. A total of 9,900 patients will be subjected to whole genome sequencing analysis with depth of 30x for normal and 120x for tumor samples, and RNA sequencing will also be conducted.

Our team (PI: Prof. Seiya Imoto of IMSUT) is building the Analysis Data Center to collect and compile a database of genomic data and clinical information of these cancer patients. The mission of the Analysis Data Center is to construct a unified analysis pipeline for primary analysis of genomic data, to collect clinical information, to build a reporting system that can be used in expert panels, to build a secure data sharing system, and to build an analysis environment that can perform advanced secondary analysis in a hybrid computational environment of on-premises and cloud.

2. Metagenome Analysis of Intestinal Microbiota

a. Unveiling viral dark matter by whole metagenome analysis of bacteriome and virome

Fujimoto K, Kimura Y, Shimohigoshi M, Satoh M, Sato S, Tremmel G, Uematsu M, Kawaguchi Y, Usui Y, Nakano Y, Hayashi T, Kashima K, Yuki Y, Yamaguchi K, Furukawa Y, Kakuta M, Akiyama Y[4], Yamaguchi R, Crowe SE[5], Ernst PB[6], Miyano S, Kiyono H, Imoto S, Uematsu S: [4]Department of Computer Science, Tokyo Institute of Technology, Japan, [5]Department of Medicine, University of California, San Diego, USA, [6]CU-UCSD Center for Mucosal Immunology, Allergy and Vaccines, University of California San Diego, USA.

The application of bacteriophages (phages) is proposed as a highly specific therapy for intestinal pathobiont elimination. However, the infectious associations between phages and bacteria in the human intestine, which is essential information for the development of phage therapies, have yet to be fully elucidated. Here, we report the intestinal viral microbiomes (viromes), together with bacterial microbiomes (bacteriomes), in 101 healthy Japanese individuals. Based on the genomic sequences of bacteriomes and viromes from the same fecal samples, the host bacteria-phage associations are illustrated for both temperate and virulent phages. To verify the usefulness of the comprehensive host bacteria-phage information, we screened Clostridioides difficile-specific phages and identified antibacterial enzymes whose activity is confirmed both in vitro and in vivo. These comprehensive metagenome analyses reveal not only host bacteria-phage associations in the human intestine but also provide vital information for the development of phage therapies against intestinal pathobionts.

b. Functional restoration of bacteriomes and viromes by fecal microbiota transplantation

Fujimoto K, Kimura Y, Allegretti JR[7], Yamamoto M, Zhang Y-Z, Katayama K, Tremmel G, Kawaguchi Y[8], Shimohigoshi M[8], Hayashi T[8], Uematsu M[8], Yamaguchi K, Furukawa Y, Akiyama Y[9], Yamaguchi R, Crowe SE[10], Ernst PB[10], Miyano S, Kiyono H, Imoto S, Uematsu S: [7]Brigham and Women's Hospital, Boston, Massachusetts, USA. [8]Osaka City University, [9]Department of Computer Science, Tokyo Institute of Technology, [10]University of California, San Diego.

Fecal microbiota transplantation (FMT) is an effective therapy for recurrent Clostridioides difficile infection (rCDI). However, the overall mechanisms underlying FMT success await comprehensive elucidation, and the safety of FMT has recently become a serious concern because of the occurrence of drug-resistant bacteremia transmitted by FMT. We investigated whether functional restoration of the bacteriomes and viromes by FMT could be an indicator of successful FMT. The human intestinal bacteriomes and viromes from 9 patients with rCDI who had undergone successful FMT and their donors were analyzed. Prophage-based and CRISPR spacer-based host bacteria–phage associations in samples from recipients before and after FMT and in donor samples were examined. The gene functions of intestinal mi-

croorganisms affected by FMT were evaluated. Metagenomic sequencing of both the viromes and bacteriomes revealed that FMT does change the characteristics of intestinal bacteriomes and viromes in recipients after FMT compared with those before FMT. In particular, many Proteobacteria, the fecal abundance of which was high before FMT, were eliminated, and the proportion of Microviridae increased in recipients. Most temperate phages also behaved in parallel with the host bacteria that were altered by FMT. Furthermore, the identification of bacterial and viral gene functions before and after FMT revealed that some distinctive pathways, including fluorobenzoate degradation and secondary bile acid biosynthesis, were significantly represented.

3. Health Medical Data Science

a. Halcyon: an accurate basecaller exploiting an encoder-decoder model with monotonic attention

Konishi H, Yamaguchi R, Yamaguchi K, Furukawa Y, Imoto S

In recent years, nanopore sequencing technology has enabled inexpensive long-read sequencing, which promises reads longer than a few thousand bases. Such long-read sequences contribute to the precise detection of structural variations and accurate haplotype phasing. However, deciphering precise DNA sequences from noisy and complicated nanopore raw signals remains a crucial demand for downstream analyses based on higher-quality nanopore sequencing, although various basecallers have been introduced to date.

To address this need, we developed a novel basecaller, Halcyon, that incorporates neural-network techniques frequently used in the field of machine translation. Our model employs monotonic-attention mechanisms to learn semantic correspondences between nucleotides and signal levels without any pre-segmentation against input signals. We evaluated performance with a human whole-genome sequencing dataset and demonstrated that Halcyon outperformed existing third-party basecallers and achieved competitive performance against the latest Oxford Nanopore Technologies' basecallers.

b. Immunogenomic pan-cancer landscape reveals immune escape mechanisms and immunoediting histories

Mizuno S[11], Yamaguchi R, Hasegawa T, Hayashi S, Fujita M[12], Zhang F[13], Koh Y[14], Lee S-Y[15], Yoon S-S[14], Shimizu E, Komura M, Fujimoto A[12], Nagai M[16], Kato M[16], Liang H[17], Miyano S, Zhang Z[13], Nakagawa H[12], Imoto S: [11]Kyushu University, [12]Riken, [13]Peking University, [14]Seoul National University Hospital, [15]Samsung SDS, [16]National Cancer Center, Japan, [17]The University of Texas MD Anderson Cancer Center, USA.

Immune reactions in the tumor microenvironment are an important hallmark of cancer, and emerging immune therapies have been proven effective against several types of cancers. To investigate cancer genome-immune interactions and the role of immunoediting or immune escape mechanisms in cancer development, we analyzed 2834 whole genome and RNA sequencing datasets across 31 distinct tumor types with respect to key immunogenomic aspects and provided comprehensive immunogenomic profiles of pan-cancers. We found that selective copy number changes in immune-related genes may contribute to immune escape. Furthermore, we developed an index of the immunoediting history of each tumor sample based on the information of mutations in exonic regions and pseudogenes and evaluated the immunoediting history of each tumor. Our immuno-genomic analyses of pan-cancers have the potential to identify a subset of tumors with immunogenicity and diverse backgrounds or intrinsic pathways associated with their immune status and immunoediting history.

c. Enhancing breakpoint resolution with deep segmentation model: a general refinement method for read-depth based structural variant callers

Zhang Y-Z, Imoto S, Miyano S, Yamaguchi R:

Read-depths (RDs) are frequently used in identifying structural variants (SVs) from sequencing data. For existing RD-based SV callers, it is difficult for them to determine breakpoints in single-nucleotide resolution due to the noisiness of RD data and the bin-based calculation. In this paper, we propose to use the deep segmentation model UNet to learn base-wise RD patterns surrounding breakpoints of known SVs. We integrate model predictions with an RD-based SV caller to enhance breakpoints in single-nucleotide resolution. We show that UNet can be trained with a small amount of data and can be applied both in-sample and cross-sample. An enhancement pipeline named RDBKE significantly increases the number of SVs with more precise breakpoints on simulated and real data.

4. COVID-19

a. A nation-wide consortium to elucidate host genetics of COVID-19 pandemic in Japan

Japan COVID-19 Task Force

To elucidate the host genetic loci affecting severity

of SARS-CoV-2 infection, or Coronavirus disease 2019 (COVID-19), is an emerging issue in the face of the current devastating pandemic. Here, we report a genome-wide association study (GWAS) of COVID-19 in a Japanese population led by the Japan COVID-19 Task Force, as one of the initial discovery GWAS studies performed on a non-European population. Enrolling a total of 2,393 cases and 3,289 controls, we not only replicated previously reported COVID-19 risk variants (e.g., LZTFL1, FOXP4, ABO, and IFNAR2), but also found a variant on 5q35 (rs60200309-A at DOCK2) that was associated with severe COVID-19 in younger (<65 years of age) patients with a genome-wide significant p-value of 1.2 × 10-8 (odds ratio = 2.01, 95% confidence interval = 1.58-2.55). This risk allele was prevalent in East Asians, including Japanese (minor allele frequency [MAF] = 0.097), but rarely found in Europeans. Cross-population Mendelian randomization analysis made a causal inference of a number of complex human traits on COVID-19. In particular, obesity had a significant impact on severe COVID-19. The presence of the population-specific risk allele underscores the need of non-European studies of COVID-19 host genetics.

b. COVID-19 risk assessment at the opening ceremony of the Tokyo 2020 Olympic Games

Murakami M[18], Miura F[19], Kitajima M[20], Fujii K[21], Yasutaka T[22], Iwasaki Y[22], Ono K[22], Shimazu Y[23], Sorano S[24], Okuda T[25], Ozaki A[26], Katayama K, Nishikawa Y[27], Kobashi Y[28], Sawano T[29], Abe T[23], Saito MM[30], Tsubokura M[18], Naito W[22], Imoto S: [18]Fukushima Medical University, [19]Ehime University, [20]Hokkaido University, [21]Kao, [22]National Institute of Advanced Industrial Science and Technology (AIST), [23]Southern TOHOKU General Hospital, [24]Nagasaki University, [25]Keio University, [26]Jyoban Hospital of Tokiwa Foundation, [27]Kyoto University, [28]Seireikai Group Hirata Central Hospital, [29]Sendai Open Hospital, [30]University of Nagasaki

The 2020 Olympic/Paralympic Games have been postponed to 2021, due to the COVID-19 pandemic. We developed a model that integrated source–environment–receptor pathways to evaluate how preventive efforts can reduce the infection risk among spectators at the opening ceremony of Tokyo Olympic Games. We simulated viral loads of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emitted from infectors through talking/coughing/sneezing and modeled temporal environmental behaviors, including virus inactivation and transfer. We performed Monte Carlo simulations to estimate the expected number of newly infected individuals with and without preventive measures, yielding the crude probability of a spectator being an infector among the 60,000 people expected to attend the opening ceremony. Two indicators, i.e., the expected number of newly infected individuals and the newly infected individuals per infector entry, were proposed to demonstrate the extent of achievable infection risk reduction levels by implementing possible preventive measures. A no-prevention scenario produced 1.5–1.7 newly infected individuals per infector entry, whereas a combination of cooperative preventive measures by organizers and the spectators achieved a 99% risk reduction, corresponding to 0.009–0.012 newly infected individuals per infector entry. The expected number of newly infected individuals was calculated as 0.005 for the combination of cooperative preventive scenarios with the crude probability of a spectator being an infector of 1 × 10−5. Based on our estimates, a combination of cooperative preventions between organizers and spectators is required to prevent a viral spread at the Tokyo Olympic/Paralympic Games. Further, under the assumption that society accepts < 10 newly infected persons traced to events held during the entire Olympic/Paralympic Games, we propose a crude probability of infectors of < 5 × 10−5 as a benchmark for the suppression of the infection. This is the first study to develop a model that can assess the infection risk among spectators due to exposure pathways at a mass gathering event.

c. COVID-19 wastewater surveillance implemented in the Tokyo 2020 Olympic and Paralympic Village

Kitajima M[31], Murakami M[32], Iwamoto R[33], Katayama H[34], Imoto S: [31]Hokkaido University, [32]Center for Infectious Disease Education and Research, Osaka University, [33]Shionogi & Co. Ltd., [34]Graduate School of Engineering, The University of Tokyo

Wastewater-based epidemiology (WBE), which has attracted attention as a COVID-19 surveillance tool,1 was implemented in the Tokyo 2020 Olympic and Paralympic Village in order to better understand COVID-19 incidence in the village.2 Between July 14 and September 8, 2021, 690 wastewater samples—361 and 329 samples collected via passive and grab sampling, respectively—were collected from manholes in the village. We collected wastewater samples, in addition to clinical data (i.e., confirmed positive cases), from seven distinct areas comprising the entire residential buildings. The wastewater samples were examined for the presence and concentration of SARS-CoV-2 RNA using a highly sensitive reverse transcription (RT)-qPCR-based detection method. We tested for SARS-CoV-2 RNA in wastewater and reported data daily to the Tokyo Organising Committee of the Olympic and Paralympic Games. The reported data were used as one of the indicators reflecting COVID-19 incidence to support judgement of the need for enhanced infection prevention measures.

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Human Genome Center

Department of Public Policy
公共政策研究分野

Professor	Kaori Muto, Ph.D.	教　授	博士(保健学)	武　藤　香　織
Associate Professor	Yusuke Inoue, Ph.D.	准教授	博士(社会健康医学)	井　上　悠　輔
Assistant Professor	Izen Ri, Ph.D.	助　教	博士(学際情報学)	李　　怡　然
Project Assistant Professor	Akiko Nagai, Ph.D.	特任助教	博士(医科学)	永　井　亜貴子

The Department of Public Policy contributes to achieving the following major missions: research ethics consultation for scientists to comply with ethical guidelines and build public trust; advancing ethical discussions and surveys surrounding COVID-19 pandemic; public policy science studies of translational research and its societal impact; and developing "minority-centered" scientific communication. Through qualitative and quantitative social science studies and policy analysis, we facilitate discussion of challenges arising from medical science advances.

1. Impacts of anxiety and socioeconomic factors on mental health in the early phases of the COVID-19 pandemic in the general population in Japan: A web-based survey

Owing to the rapid spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic worldwide, individuals experience considerable psychological distress daily. The present study aimed to clarify the prevalence of psychological distress and determine the population most affected by risk factors such as the pandemic, socioeconomic status (SES), and lifestyle-related factors causing psychological distress in the early phases of the pandemic in Japan. This study was conducted via a web-based survey using quota sampling to ensure representativeness of the Japanese population aged 20–64 years. A cross-sectional study of 11,342 participants (5,734 males and 5,608 females) was conducted using a self-administered questionnaire that included the Japanese version of the Kessler 6 Psychological Distress Scale (K6) and questions related to the pandemic, SES, and lifestyle. The prevalence of psychological distress, represented by a K6 score of 5 or more, was 50.3% among males and 52.6% among females. Both males and females with annual household incomes less than 2 million yen and males aged in their twenties had significantly higher K6 scores than those with annual household incomes above 2 million yen and males aged over 30 years. Binary logistic regression analyses found pandemic-related factors such as medical history, inability to undergo clinical tests immediately, having trouble in daily life, unavailability of groceries, new work style, and vague anxiety; SES-related factors such as lesser income; and lifestyle-related factors such as insufficient rest, sleep, and nutritious meals to be significantly related to psychological distress. Psychological distress was more prevalent among people with low income and in younger generations than among other groups. There is an urgent need to provide financial, medical, and social support to those affected by the coronavirus disease 2019 (COVID-19) pandemic.

2. SARS-CoV-2 Human Challenge Trials: Rethinking the Recruitment of Healthy Young Adults First

In the midst of the ongoing Covid-19 pandemic, researchers across the globe are still working to devel-

op effective vaccines. To expedite this process even further, human challenge trials have been proposed by the World Health Organization (WHO) as an alternative to conventional approaches. In such trials, healthy volunteers are deliberately infected with the pathogen of interest, enabling scientists to study the infection process and facilitate further research on treatments or prophylactics, including vaccines. While human challenge trials would offer a collective benefit to society, minimizing the risks is always difficult. Ethical controversy thus inevitably surrounds these trials. Typically, healthy young adults are recruited to serve as the first candidate subjects for human challenge trials because they are generally considered to represent a low-risk population. Here, we present three reasons for doubt about this healthy-young-adults-first criterion and give justification for also recruiting healthy older adults (or not-young adults), meaning those over 30 years of age, to participate in such trials for SARS-CoV-2.

3. Proactive Engagement of the Expert Meeting in Managing the Early Phase of the COVID-19 Epidemic, Japan, February–June 2020

To deal with the risk of emerging diseases with many unknowns, close and timely collaboration and communication between science experts and policymakers are crucial to developing and implementing an effective science-based intervention strategy. The Expert Meeting, an ad hoc medical advisory body, was established in February 2020 to advise Japan's COVID-19 Response Headquarters. The group played an important role in the policymaking process, promoting timely situation awareness and developing science-based proposals on interventions that were promptly reflected in government actions. However, this expert group may have been overly proactive in taking on the government's role in crisis management. For the next stage of managing the coronavirus disease pandemic and future pandemics, the respective roles of the government and its advisory bodies need to be clearly defined. Leadership and strategic risk communication by the government are key.

4. Cause-specific mortality rates in patients with diabetes according to comorbid macro- and microvascular complications: BioBank Japan Cohort

This study aimed to compare cause-specific mortality rates in patients with type 2 diabetes with and without various vascular complications. In Japanese hospitals, we followed up 30 834 patients with a mean age of 64.4 (standard deviation [SD]: 11.1) years. Patients were followed up from 2003 to 2007 for a median of 7.5 (interquartile range: 6.1-9.7) years. We calculated cause-specific mortality rates (number of deaths/1000 person-years) and confounder-adjusted hazard ratios in patients with macrovascular disease and in those with diabetic nephropathy, neuropathy and retinopathy, allowing for overlap of complications. All-cause mortality rate was highest (51.4) in the nephropathy group, followed by the macrovascular disease group (45.2), the neuropathy group (39.5), the retinopathy group (38.7) and the nonvascular complication group (18.1). In the nephropathy group, morality rates of ischaemic heart, cerebrovascular, and infectious diseases and cancer were also highest among the groups. However, the cancer mortality rate was similar among the vascular complication groups. Relative to the nonvascular complication group, covariate-adjusted hazard ratios for ischaemic heart and cerebrovascular disease mortality were triple to quadruple in the macro- and microvascular complication groups. All-cause mortality rates rose exponentially according to age. Highest risks of all-cause, cancer, and ischaemic heart, infectious, and cerebrovascular disease mortality were determined in Japanese patients with diabetic nephropathy. Although cancer is the primary cause of death in Japanese patients with diabetes, cancer mortality rates are similar among those with and without vascular complications.

5. Ethical, legal and social implications of human genome studies in radiation research: a workshop report for studies on atomic bomb survivors at the Radiation Effects Research Foundation

The Radiation Effects Research Foundation (RERF) is the primary organization in Japan dedicated to studying the health consequences of the Hiroshima and Nagasaki atomic bombings in World War II. In December 2020, RERF held a virtual international workshop on the ethical, legal and social implications (ELSI) of genome studies. In this workshop, the ELSI considerations of future human genome studies on radiation research including atomic bomb survivors and their families were discussed. Since genome sequencing (GS) is now practical and affordable, RERF now plans GS of parents/child trios to examine genetic effects of atomic bomb radiation. As such studies may engender some novel risks and benefits, ethics review and engagement with families (including consent) need to be considered. These include protection of individual privacy, use of samples from deceased prior participants, return of results to the participants, public sharing of genome data and advance science and social welfare. Specifically with regard to social welfare, the results of such studies may have implications for public and government decision-making regarding social benefits of victims and other important questions. Based on these broad-ranging discussions we have developed the following concepts to guide this work: "trust," "compromise" and "relationship building," inclusive of the concerned stakeholders,

scientific aims and Japanese society at large. We conclude that in order to realize, establish and maintain these concepts, it is essential to put procedures into place to ensure the successful, consensus-based implementation of the RERF studies.

6. Ethical Issues: Overview in Genomic Analysis and Clinical Context

We discuss ethical, legal, and social issues (ELSI) centered around hereditary breast and ovarian cancer syndrome (HBOC). We describe ethical considerations in the context of decision-making on genetic testing, debates on incidental/secondary findings (IFs/SFs), and global trends in clinical and/or genetic data sharing, including with patients and their family members. From the perspective of clinical ethics of cancer diagnosis and treatment, we introduce the importance of decision-making and care based on the shared decision-making (SDM) approach and practical points in prophylactic surgery. We also discuss dilemmas that arise regarding confidentiality between medical professionals and their patients. This includes disclosure of genetic information with genetic relatives, and challenges in family communication, in which carefully assessed and encouraging support may be needed for patients and family members.

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Human Genome Center

Division of Medical Data Informatics
医療データ情報学分野

Professor	Tetsuo Shibuya, Ph.D.	教　授	博士(理学)　渋　谷　哲　朗
Project Assistant Professor	Robert Daniel Barish, Ph.D.	特任助教	博士(学術)　ロバート　ダニエル　バリッシュ

The objective of Division of Medical Data Informatics is to develop fundamental data informatics technologies for medical data, including algorithm theory, big data technologies, artificial intelligence, data mining, and privacy preserving technologies. Medical data, especially genome data are increasing exponentially from basics to clinical research in medical science. Our aim is to innovate medical science with novel data informatics solutions.

1. Development of Artificial Intelligence Technologies for Biomedical Data

a. Natural Language Processing Methods for International Medical Text Databases

Arda Akdemir[1], Tetsuo Shibuya[1], Tunga Güngör[2]: [1]Division of Medical Data Informtatics, Institute of Medical Science, the University of Tokyo, [2]Department of Computer Engineering, Bogaziçi University

Deep language models such as BERT pretrained on large-scale datasets have enabled remarkable progress in a wide range of NLP tasks and became the standard approach for many languages. However, an in-depth understanding of the effect of using these models is still missing for less spoken languages. This study gives a comprehensive analysis of using the BERT model for languages with rich morphology. We experimented with cross-lingual, multilingual, and monolingual BERT models and three non-BERT-based models. We evaluated these models on five morphologically rich languages (Finnish, Czech, Hungarian, Turkish, Japanese) and the English language. Evaluated on Dependency Parsing (DEP) and Named Entity Recognition (NER) tasks, which benefit highly from morphological information, BERT-based models consistently outperformed other approaches. Results revealed that the effects of using BERT-based models significantly differ across languages. Moreover, our analysis provided various critical findings of multi-task learning (MTL), transfer learning, and external features in different settings. We further verified these findings on noisy datasets for the Sentiment Analysis task as a case study. Finally, the propose BERT-based model achieved new state-of-the-art results on both DEP and NER tasks for the Turkish language.

High-quality training datasets are critical for building successful Machine Learning (ML) based NLP systems. However, these datasets are not always available in low-resource contexts such as the biomedical domain. Here, selecting relevant training data is as important as the choice of the ML model. We propose UDON: Unsupervised Data selectiON for biomedical entity recognition using domain-specific pretrained Language Models (LMs). We first show that pretrained LMs succeed at implicitly learning the differences between datasets without any supervision, and then use these models to select relevant data instances. Next, we evaluate the propose methods for entity recognition on seven biomedical datasets and one news domain dataset using four LMs and three selection methods. Our results show that using pretrained domain-specific LMs for data selection outperforms all other approaches. Finally,

we use domain classification as an auxiliary task for pretraining the neural network on the in-domain dataset and show it yields further improvements.

c. Feature Selection Methods for Cancer Microarray Data Classification

Zixuan Wang[1], Yi Zhou[3], Tatsuya Takagi[3], Yu-shi Tian[3], Tetsuo Shibuya[1]: [3]Graduate School of Pharmaceutical Sciences, Osaka University

Gene microarray data is a high-profile topic in the field of bioinformatics, which can help researchers to explore the correlation between gene expression and cancer. The establishment of classification models can be applied to identify the gene expression patterns of different cancer. However, it is a challenging task that microarray data often contains thousands of genes and very small number of samples. Here, we propose a novel Genetic Algorithm (GA) feature selection method embedded a manifold learning algorithm, Isometric feature mapping (Isomap). The purposes of this method are: (1) Select the subset of cancer-related genes; (2) Improve the performance of cancer classification prediction. Isomap is embedded into fitness function of GA to reduce the bias caused by the potential nonlinear structure of microarray data. Considering the randomness of GA, we also adopt a framework that treats genes that are selected multiple times as informative genes, which guarantees that the probability of being selected due to randomness is negligible. Comprehensive results of eight benchmark cancer microarray datasets show that our proposed method can get the highest ranking-score, that is, using fewer genes to get almost the same or even higher classification accuracy.

2. Development of Privacy Preserving Technologies for Medical Data

a. Differential Privacy Methods for Publishing Medical Data Statistics

Akito Yamamoto[1], Tetsuo Shibuya[1]

To achieve the provision of personalized medicine, it is very important to investigate the relationship between diseases and human genomes. For this purpose, large-scale genetic studies such as genome-wide association studies are often conducted, but there is a risk of identifying individuals if the statistics are released as they are.

i) Publishing statistics

Genome-wide association studies, which are large-scale genetic statistical analyses, often involve tests with contingency tables. However, if the statistics obtained by these tests are made public as they are, sensitive information of individuals could be leaked. Existing studies have proposed privacy-preserving methods for statistics in the χ^2 test with a 3×2 contingency table, but they do not cover all the tests used in association studies. In addition, existing methods for releasing differentially private P-values are not practical. We propose methods for releasing statistics in the χ^2 test, the Fisher's exact test and the Cochran–Armitage's trend test while preserving both personal privacy and utility. Our methods for releasing P-values are the first to achieve practicality under the concept of differential privacy by considering their base 10 logarithms. We make theoretical guarantees by showing the sensitivity of the above statistics. From our experimental results, we evaluate the utility of the proposed methods and show appropriate thresholds with high accuracy for using the private statistics in actual tests.

We also propose efficient and practical privacy-preserving methods using the concept of differential privacy for linkage analysis with a transmission disequilibrium test (TDT). We focus on the case of two affected children in one family, and present differentially private data sharing methods based on three statistics, which are the TDT statistic, haplotype-based statistic, and combined statistic of these two. First, we show the sensitivities of each statistic and present the algorithm using the Laplace mechanism. Then, for the exponential mechanism, we adopt the shortest Hamming distance score as the score function and propose exact and approximation algorithms to find the scores. In our experiments, we measure the run time of each algorithm to show that it is feasible even on a large database.

ii) Methods for Larger Cohort

We furthermore propose new efficient differentially private methods for a transmission disequilibrium test, which is a family-based association test. Existing methods are computationally intensive and take a long time even for a small cohort. Moreover, for approximation methods, sensitivity of the obtained values is not guaranteed. We present an exact algorithm with a time complexity of O(nm) for a dataset containing n families and m single nucleotide polymorphisms (SNPs). We also propose an approximation algorithm that is faster than the exact one and prove that the obtained scores' sensitivity is 1. From our experimental results, we demonstrated that our exact algorithm is 10,000 times faster than existing methods for a small cohort with 5,000 SNPs. The results also indicate that the proposed method that can be applied to a large cohort. In addition, we examined a suitable dataset to apply our approximation algorithm.

b. Development of Fair and Differential Private Machine Learning Methods

Deng Jiaming[1], Tetsuo Shibuya[1]

Machine learning methods have been widely utilized in classification tasks. Along with their enhancement, privacy and fairness concerns on sensitive data have also draw much attention. However, they are mostly studied in isolation. The trade-offs between them are not simply mutual incompatible. Although it is quite challenging to achieve both requirements due to the disparate goals of fairness and privacy, it is worth diving into exploiting the potential of simultaneously achieving privacy and fairness.

For this issue, we developed a new model-training framework which offers strict privacy guarantees for sensitive data, Private Aggregation of Teacher Ensembles, or PATE, and develop differentially private and fair classification models by incorporating domain adaptation algorithm based on adversarial learning in a united manner. The adversarial neural network serves as an additional term towards the objective function in order to enforce fair classification. The functional mechanism brings randomness to both teacher and student models in PATE which provides strict privacy guarantees.

3. Development of Biomedical Database Technologies

a. Development of Compression Algorithms for Next Generation Sequencer Data

Kazushi Kitaya[1], Tetsuo Shibuya[1]

A set of *k*-mers is used in many bioinformatics tasks, such as genome assembly, individual genome mapping, etc., but they require enormous amount of memory/storage space to store. Thus, much work has been done on methods to efficiently represent or compress a single set of *k*-mers. However, methods for compressing multiple k-mer sets have been less studied in spite of their obvious benefits for researchers and genome-related database maintainers. We developed an algorithm to compress multiple *k*-mer sets, based on iterative SPSS (spectrum-preserving string sets) decomposition. In the experiments with 3292 *k*-mer sets constructed from *E. coli* whole-genome sequencing data and 2555 *k*-mer sets constructed from human RNA-Seq data, our algorithm reduces the compressed file sizes by 34.7% and 13.2% respectively compared to the state-of-the-art colored de Bruijn graph representations. Moreover, our method uses less memory than the colored de Bruijn graph method. We also introduce various methods to make the compression algorithm efficient in terms of time and memory, *e.g.*, a parallelizable small-weight SPSS construction algorithm.

b. Computational Theory for Graph/Network Databases

Robert Barish[1], Tetsuo Shibuya[1]

We consider what we denote the Embedded Agent Limited Visibility Teleportation Maze (EALVTM) problem of efficiently utilizing a player-controlled agent, with distance-limited vision, to discover and subsequently navigate to a target of interest by moving along the edges of a rectangular or triangular mesh. Here, we prove an NP-hardness result for the optimization version of EALVTM where the objective is to minimize the total number of edge-wise hops, determine a set of cases where the problem of finding an object on a given mesh becomes Fixed-Parameter Tractable (FPT), and show how recent advances in low-distortion stochastic embeddings of higher-genus graphs in the plane can be used to extend existing planar graph coverage and path search algorithms to treat the EALVTM problem on meshes embedded in surfaces having bounded orientable or non-orientable genus.

c. Integrating Viruses and Cellular Organisms for Pathway Maps

Mari Ishiguro-Watanabe[1], Minoru Kanehisa[4]: [4]Institute for Chemical Research, Kyoto University.

KEGG is a manually curated resource on systems, genomic, chemical and health information, with KEGG mapping tools which enable understanding of cellular and organism-level functions. Since the introduction of the KEGG NETWORK database, various diseases have been associated with network variants, which are perturbed molecular networks caused by human gene variants, viruses, other pathogens and environmental factors. The network variation maps are created as aligned sets of related networks showing, for example, how different viruses inhibit or activate specific cellular signaling pathways. The KEGG pathway maps are now integrated with network variation maps in the NETWORK database, as well as with conserved functional units of KEGG modules and reaction modules in the MODULE database. The KO database for functional orthologs continues to be improved and virus KOs are being expanded for better understanding of virus-cell interactions and for enabling prediction of viral perturbations.

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Human Genome Center

Division of Metagenome Medicine
メタゲノム医学分野

Project Professor	Satoshi Uematsu, M.D., Ph.D.	特任教授　博士（医学）	植　松　　智
Project Assistant Professor	Kosuke Fujimoto, M.D., Ph.D.	特任助教　博士（医学）	藤　本　康　介

Abnormal compositions of intestinal microbiota have been reported to be associated with various diseases. We analyze intestinal bacteriome and virome in various diseases and search for "pathobiont" that causes the diseases. By making use of bioinformatics, we are constructing an analysis pipeline for intestinal microbiome, conducting comprehensive metagenomic analysis, and developing phage therapy for the specific control of pathobionts.

1. Analysis of intestinal microbiota in disease.

Kosuke Fujimoto[1], Seiya Imoto[2] and Satoshi Uematsu[1]

[1]Division of Metagenome Medicine, Human Genome Center, The Institute of Medical Science, The University of Tokyo. [2]Division of Health Medical Inteligence, Human Genome Center, The Institute of Medical Science, The University of Tokyo.

In recent years, not only in intestinal diseases such as inflammatory bowel disease, but also in autoimmune diseases, diabetes, cardiovascular diseases, and autism, dysbiosis have been detected. It is now clear that dysbiosis is closely related to the pathogenesis of the diseases. It is expected to treat the disease by improving dysbiosis.Fecal microbiota transplantation (FMT) is an effective therapy for recurrent Clostridioides difficile infection (rCDI). However, the overall mechanisms underlying FMT success await comprehensive elucidation, and the safety of FMT has recently become a serious concern because of the occurrence of drug-resistant bacteremia transmitted by FMT. We investigated whether functional restoration of the bacteriomes and viromes by FMT could be an indicator of successful FMT.The human intestinal bacteriomes and viromes from 9 patients with rCDI who had undergone successful FMT and their donors were analyzed. Prophage-based and CRISPR spacer-based host bacteria-phage associations in samples from recipients before and after FMT and in donor samples were examined. The gene functions of intestinal microorganisms affected by FMT were evaluated. Metagenomic sequencing of both the viromes and bacteriomes revealed that FMT does change the characteristics of intestinal bacteriomes and viromes in recipients after FMT compared with those before FMT. In particular, many Proteobacteria, the fecal abundance of which was high before FMT, were eliminated, and the proportion of Microviridae increased in recipients. Most temperate phages also behaved in parallel with the host bacteria that were altered by FMT. Furthermore, the identification of bacterial and viral gene functions before and after FMT revealed that some distinctive pathways, including fluorobenzoate degradation and secondary bile acid biosynthesis, were significantly represented. The coordinated action of phages and their host bacteria restored the recipients' intestinal flora. These findings show that the restoration of intestinal microflora functions reflects the success of FMT. In the future, we will conduct a comprehensive metagenomic analysis of feces from patients with Crohn's disease and neurodegenerative disorders to investigate the role of dysbiosis in pathological mechanisms.

2. Development of treatments for dysbiosis-related diseases by comprehensive metagenomic analysis

Our intestinal tract carries a lot of bacteria in the lumen as the resident microorganism. In addition to resident bacteria, viruses are also present in our intestinal tract, most of which are bacteriophages. However, it is still unclear what kind of bacteriophage exist in our intestinal tract, and what kind of bacteria they infect with. As one of the reasons, isolation of viral nucleic acids and preparation of libraries have not been established. Since conserved sequence such as 16s rRNA gene do not exist in virus, whole genome analysis is necessary. Even if comprehensive whole genome analysis of intestinal viruses were performed, most of the sequence fragments couldn't be classified by homology search due to the insufficient public databases. Thus, virome analysis is relatively difficult compared with bacteiome analysis and this situation is expressed by the word "vral dark matter". We have developed the isolation method of intestinal viruses. We also have generated analysis pipeline of metagenome analysis of viral genome and the method to analyze host-parasite association identified based on the shotgun sequencing data of the bacterial flora and viral plexus. We are now generating the foundation for next-generation phage therapy which uses phage-derived enzymes to kill bacteria and artificial phage therapy for the elimination of intestinal pathobionts.

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Center for Experimental Medicine and Systems Biology

Division of Stem Cell Pathology
先進病態モデル研究分野

Professor	Yasuhiro Yamada, M.D., Ph.D.	教　授	博士(医学)	山　田　泰　広
Assistant Professor	Sho Ohta, Ph.D.	助　教	博士(生命科学)	太　田　　翔
Assistant Professor	Nao Sankoda, M.D., Ph.D.	助　教	博士(医学)	三小田　　直

Epigenetic regulation plays a critical role for the cellular differentiation, the stable maintenance of cellular identity, and the reprogramming process. Accumulating evidence suggests that epigenetic abnormalities represented by abnormal DNA methylation have been involved in various diseases as well. We are interested in unveiling epigenetic regulation in the cellular differentiation, the maintenance of cellular identity, and the pathogenesis including age-related diseases such as cancer. Particularly, taking advantage of reprogramming technology to actively alter epigenetic regulation, we are investigating the role of epigenetic regulation on cancer development, maintenance, and progression. Finally, we will try to develop a novel approach targeting epigenetic regulation to treat cancer patients.

1. DMRT1-mediated *in vivo* reprogramming drives development of cancer resembling human germ cell tumors with features of totipotency

Jumpei Taguchi, Hirofumi Shibata[1,2], Mio Kabata[1], Masaki Kato[3,4], Kei Fukuda[3], Akito Tanaka[1], Sho Ohta, Tomoyo Ukai, Kanae Mitsunaga[1], Yosuke Yamada[1,5], So Nagaoka[6,7], Sho Yamazawa[8], Kotaro Ohnishi[1,9], Knut Woltjen[1], Tetsuo Ushiku[8], Manabu Ozawa[10], Mitinori Saitou[1,6,11], Yoichi Shinkai[3], Takuya Yamamoto[1,11,12,13] and Yasuhiro Yamada[13]

[1]Department of Life Science Frontiers, Center for iPS Cell Research and Application (CiRA), Kyoto University.
[2]Department of Otolaryngology, Gifu University Graduate School of Medicine.
[3]Cellular Memory Laboratory, RIKEN Cluster for Pioneering Research.
[4]Laboratory for Transcriptome Technology, RIKEN Center for Integrative Medical Sciences.
[5]Department of Diagnostic Pathology, Kyoto University Hospital.
[6]Department of Anatomy and Cell Biology, Graduate School of Medicine, Kyoto University.
[7]Department of Embryology, Nara Medical University.
[8]Department of Pathology, Graduate School of Medicine, The University of Tokyo.
[9]Department of Gastroenterology/Internal Medicine, Gifu University Graduate School of Medicine.
[10]Laboratory of Reproductive Systems Biology, Center for Experimental Medicine and Systems Biology, Institute of Medical Science, The University of Tokyo.
[11]Institute for the Advanced Study of Human Biology (WPI-ASHBi), Kyoto University.
[12]Medical-risk Avoidance Based on iPS Cells Team, RIKEN Center for Advanced Intelligence Project (AIP).
[13]AMED-CREST, AMED.

In vivo reprogramming provokes a wide range of cell fate conversion. We found that *in vivo* induction of higher levels of OSKM in mouse somatic cells leads to increased expression of primordial germ cell (PGC)-related genes and provokes genome-wide erasure of genomic imprinting, which takes place exclusively in PGCs. *In vivo* OSKM reprogramming caused propagation of OCT4/NANOG-positive cells, resulting in development of cancer that resembled human germ

cell tumors. Like a subgroup of germ cell tumors, propagated tumor cells could differentiate into trophoblasts. Moreover, these tumor cells gave rise to induced pluripotent stem cells (iPSCs) with expanded differentiation potential that could contribute to adult mice. DMRT1, which is expressed in PGCs, drove the reprogramming and propagation of the tumor cells *in vivo*. Furthermore, DMRT1-mediated reprogramming is associated with trophoblast competence of the reprogrammed cells and provides a therapeutic target for germ cell tumors. These results reveal a novel route for somatic cell reprogramming and underscore the impact of reprogramming in development of germ cell tumors. Furthermore, our findings may have implications regarding acquisition of totipotency-like features by somatic cells.

2. Epithelial expression of Gata4 and Sox2 regulates specification of the squamous-columnar junction via MAPK/ERK signaling in mice

Nao Sankoda [1,14], Wataru Tanabe [1, 15], Akito Tanaka [1], Hirofumi Shibata [2], Knut Woltjen [1, 16], Tsutomu Chiba [15], Hironori Haga [5], Yoshiharu Sakai [14], Masaki Mandai [17], Takuya Yamamoto [1,11,12,13], Yasuhiro Yamada [13], Shinji Uemoto [14], Yoshiya Kawaguchi [1]

[14]Department of Surgery, Kyoto University Graduate School of Medicine.

[15]Department of Gastroenterology, Kyoto University Graduate School of Medicine.

[16]Hakubi Center for Advanced Research, Kyoto University.

[17] Department of Gynecology and Obstetrics, Kyoto University Hospital.

The squamous-columnar junction (SCJ) is a boundary consisting of precisely positioned transitional epithelium between the squamous and columnar epithelium. Transitional epithelium is a hotspot for precancerous lesions, and is therefore clinically important; however, the origins and physiological properties of transitional epithelium have not been fully elucidated. Here, by using mouse genetics, lineage tracing, and organoid culture, we examine the development of the SCJ in the mouse stomach, and thus define the unique features of transitional epithelium. We find that two transcription factors, encoded by Sox2 and Gata4, specify primitive transitional epithelium into squamous and columnar epithelium. The proximal-distal segregation of Sox2 and Gata4 expression establishes the boundary of the unspecified transitional epithelium between committed squamous and columnar epithelium. Mechanistically, Gata4-mediated expression of the morphogen Fgf10 in the distal stomach and Sox2-mediated Fgfr2 expression in the proximal stomach induce the intermediate regional activation of MAPK/ERK, which prevents the differentiation of transitional epithelial cells within the SCJ boundary. Our results have implications for tissue regeneration and tumorigenesis, which are related to the SCJ.

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Center for Experimental Medicine and Systems Biology

Laboratory of Innate Immunity
自然免疫研究分野

Professor Kensuke Miyake, M.D., Ph.D.

教 授 医学博士 三 宅 健 介

Pathogen sensors, such as Toll-like receptor (TLR), play sentinel roles in detecting pathogenic ligands during infection and induce both innate and acquired immune responses. Meanwhile, excessive TLR responses are strongly associated with fatal diseases such as septic shock and autoimmune diseases. For this reason, immune system must strictly control TLR responses to avoid disruption of homeostasis. However, molecular mechanisms involved in TLR regulation are not fully elucidated. We have previously shown that TLRs are regulated by various TLR associating molecules including MD-2, PRAT4A and Unc93B1. Our goal is to uncover molecular mechanism that is indispensable for appropriate TLR responses using genetically engineered mice.

1. Targeting the nucleic acids-sensing TLRs for therapeutic intervention in autoimmune diseases

Yuji Motoi[1], Ryutaro Fukui[1], Takuma Shibata[1], Kensuke Miyake[1,2]
[1]Division of Innate Immunity, Department of Microbiology and Immunology, [2]Laboratory of Innate Immunity, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minatoku, TOKYO1208-8639, Japan.

TLR7 senses microbial-derived RNA in endolysosome, but can also erroneously respond to self-derived RNA. In fact, it has been reported that TLR7-dependent signaling promote autoimmune diseases. Thus, TLR7 can be therapeutic target. Although antibodies (Abs) are powerful tools for therapeutic intervention, TLR7 has been excluded from targets for Ab-mediated intervention because of its lack of cell surface expression. Despite this expectation, we found an anti-TLR7 Ab dose-dependently inhibits TLR7 responses in dendritic cells, macrophages and B cells. For this reason, we evaluated the therapeutic effect of anti-TLR7 Ab in *Unc93b1*$^{D34A/D34A}$ mice that cause thrombocytopenia, splenomegaly and chronic active hepatitis due to TLR7 hyper-responsiveness, and found that thrombocytopenia in *Unc93b1*$^{D34A/D34A}$ mice was significantly improved by the treatment with anti-TLR7 mAb. Furthermore, splenomegaly and hepatitis in mice treated with the anti-TLR7 mAb were also significantly remedy compared with control antibody.

On basis of these results, we established anti-human TLR7 Ab for blocking human TLR7 responses in vitro. Moreover, we generated human TLR7 transgenic (huTLR7 Tg) mice. We plan to use HuTLR7 Tg mice to evaluate the effects of anti-human TLR7 Ab *in vivo*.

In addition, TLR8 also recognize mouse TLR7 ligands in human and is involved in exacerbation of Rheumatoid Arthritis. Thus, in case of human disease, the anti-human TLR8 Ab that inhibits human TLR8 responses might work in clinical application. For this reason, we also constructed both anti-human TLR8 Abs and human TLR8 transgenic mice to verify our hypothesis.

2. Skewed endosomal RNA responses from TLR7 to TLR3 in RNase T2-deficient macrophages

Kaiwen Liu[1, *], Ryota Sato[1, *], Takuma Shibata[1], Ryosuke Hiranuma[1], Tatjana Reuter[1, 4, 5], Ryutaro Fukui[1], Yun Zhang[1], Takeshi Ichinohe[6], Manabu Ozawa[3], Nobuaki Yoshida[3], Eicke Latz[4, 5, 7], Kensuke Miyake[1, 2, **]

[1]Division of Innate Immunity, Department of Microbiology and Immunology, [2]Laboratory of Innate Immunity, Center for Experimental Medicine and Systems Biology, [3]Laboratory of Development Genetics, Laboratory of Reproductive Systems Biology, Center for Experimental Medicine and Systems Biology, [6]Department of Infectious Disease Control, International Research Center for Infectious Diseases, Institute of Medical Science, The University of Tokyo, Tokyo, 108-8639, Japan. [4]Institute of Innate Immunity, Biomedical Center, Venusberg-Campus, University of Bonn, Bonn, Germany.

[5]German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany. [7]Department of Infectious Diseases and Immunology, UMass Medical School, Worcester, MA, USA.

RNase T2, a ubiquitously expressed RNase, degrades RNAs in the endosomal compartments. RNA sensors, double-stranded RNA (dsRNA)-sensing TLR3 and single-stranded RNA (ssRNA)-sensing TLR7, are localized in the endosomal compartment in mouse macrophages. We here studied the role of RNase T2 in TLR3 and TLR7 responses in macrophages. Macrophages expressed RNase T2 and a member of the RNase A family RNase 4. RNase T2 was also expressed in plasmacytoid and conventional dendritic cells. Treatment with dsRNAs or type I interferon (IFN) upregulated expression of RNase T2 but not RNase 4. RNase T2-deficiency in macrophages upregulated TLR3 responses but impaired TLR7 responses. Mechanistically, RNase T2 degraded both ds- and ssRNAs *in vitro*, and its mutants showed a positive correlation between RNA degradation and the rescue of altered TLR3 and TLR7 responses. H122A and C188R RNase T2 mutations, not H69A and E118V mutations, impaired both RNA degradation and the rescue of altered TLR3 and TLR7 responses. RNase T2 in bone marrow-derived macrophages was broadly distributed from early endosomes to lysosomes, and colocalized with the internalized TLR3 ligand poly(I:C). These results suggest that RNase T2-dependent RNA degradation in endosomes/lysosomes negatively and positively regulates TLR3 and TLR7 responses, respectively, in macrophages.

3. The impact of cell maturation and tissue microenvironments on the expression of endosomal Toll-like receptors in monocytes and macrophages

Ryota Sato [1,] Tatjana Reuter [1 2], Ryosuke Hiranuma [1], Takuma Shibata [1], Ryutaro Fukui [1], Yuji Motoi [1], Yusuke Murakami [1], Hiroki Tsukamoto [3 4], Satoshi Yamazaki [5], Kaiwen Liu [1], Shin-Ichiroh Saitoh [1], Eicke Latz [2 6 7], Kensuke Miyake [1,8]

[1]Division of Innate Immunity, Department of Microbiology and Immunology, [2] Institute of Innate Immunity, Biomedical Center, Venusberg-Campus, University of Bonn, Bonn, Germany. [3]Laboratory of Oncology, Pharmacy Practice and Sciences, Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai, Japan. [4]Department of Pharmaceutical Sciences, School of Pharmacy at Fukuoka, International University of Health and Welfare, Fukuoka, Japan. [5]Laboratory of Stem Cell Therapy, Faculty of Medicine, University of Tsukuba, Tsukuba, Japan. [6]German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany. [7]Department of Infectious Diseases and Immunology, UMass Medical School, Worcester, MA, USA. [8]Laboratory of Innate Immunity, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

Toll-like receptors (TLRs) impact myeloid cell responsiveness to environmental cues such as pathogen components and metabolites. Although TLR protein expression in monocytes and tissue macrophages is thought to be optimized for microenvironments in each tissue, a comprehensive study has not been reported. We here examined protein expression of endogenous TLRs in tissue-resident myeloid cells. Neutrophils in peripheral blood, spleen, liver and lung expressed TLR2, TLR4 and TLR5 in all tissues. Ly6C+ MHC II– classical monocytes mature into Ly6C– MHC II+ monocyte-derived dendritic cells (moDCs) or Ly6C– MHC II– patrolling monocytes. These subsets were found in all the tissues studied. TLR2 and TLR4 were displayed on all of these subsets, regardless of location. In contrast, expression of endosomal TLRs did vary with tissues and subsets. moDCs expressed TLR9, but much less TLR7. In contrast, TLR7, not TLR3 or TLR9, was highly expressed in classical and patrolling monocytes. Tissue macrophages such as red pulp macrophages in the spleen, Kupffer cells in the liver, microglia in the brain, alveolar macrophages in the lung and adipose tissue macrophages all expressed TLR2, TLR4 and TLR3. TLR7 was also expressed in these tissue macrophages except Kupffer cells in the liver. TLR9 expression in tissue macrophages was much lower or hard to detect. These results suggest that expression of endosomal TLRs in myeloid cells is influenced by their differentiation status and tissue-specific microenvironments.

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Center for Experimental Medicine and Systems Biology

Laboratory of Reproductive Systems Biology
生殖システム研究分野

Project Professor Masahito Ikawa, Ph.D.
Associate Professor Manabu Ozawa, Ph.D.

特任教授 博士(薬学) 伊川正人
准教授 博士(農学) 小沢学

In the "post-genome project era," genetically modified animals play a key role in basic molecular biological investigations and act as models of human disease. Our laboratory studies the mechanisms underlying the mammalian reproductive system in gene-manipulated mice. We are the first group in the world to generate transgenic mice expressing GFP throughout the body (Green mice). We also established the ES cells that give green fluorescent spermatozoa to trace their movement and acrosome reaction during fertilization. Another tool invented in our laboratory is the placenta-specific gene manipulation system using lentiviral (LV) vectors. Using these techniques, we are trying to elucidate the mechanism underlying gametogenesis, fertilization, implantation, and placentation. Our recent interest is using the CRISPR/Cas9 system as a genome-editing tool. The combination of GWAS studies with genome editing will pave the way to understand and control human fertility problems.

1. Rubicon prevents autophagic degradation of GATA4 to promote Sertoli cell function

Tadashi Yamamuro [1], Shuhei Nakamura [1, 2, 3], Yu Yamano [2], Tsutomu Endo [4], Kyosuke Yanagawa [1, 5], Ayaka Tokumura [1], Takafumi Matsumura [4], Kiyonori Kobayashi [4], Hideto Mori [6, 7], Yusuke Enokidani [1], Gota Yoshida [1], Hitomi Imoto [1, 2], Tsuyoshi Kawabata [1, 2, 8], Maho Hamasaki [1, 2], Akiko Kuma [1, 2], Sohei Kuribayashi [9], Kentaro Takezawa [9], Yuki Okada [10], Manabu Ozawa , Shinichiro Fukuhara [9], Takashi Shinohara [11], Masahito Ikawa [4] , Tamotsu Yoshimori [1, 2, 12]

[1]Department of Genetics, Graduate School of Medicine, Osaka University.
[2]Laboratory of Intracellular Membrane Dynamics, Graduate school of Frontier Biosciences, Osaka University.[3]Institute for Advanced Co-Creation Studies, Osaka University.[4]Research Institute for Microbial Diseases, Osaka University.[5]Department of Cardiovascular Medicine, Graduate School of Medicine, Osaka University.[6]Institute for Advanced Biosciences, Keio University.[7]Graduate School of Media and Governance, Keio University.[8]Department of Stem Cell Biology, Atomic Bomb Disease Institute, Nagasaki University.[9]Department of Urology, Graduate School of Medicine, Osaka University.[10]Laboratory of Pathology and Development, The Institute for Quantitative Biosciences, The University of Tokyo.[11] Department of Molecular Genetics, Graduate School of Medicine, Kyoto University.[12] Integrated Frontier Research for Medical Science Division, Institute for Open and Transdisciplinary Research Initiatives (OTRI), Osaka University.

Autophagy degrades unnecessary proteins or damaged organelles to maintain cellular function. Therefore, autophagy has a preventive role against various diseases including hepatic disorders, neurodegenerative diseases, and cancer. Although autophagy in germ cells or Sertoli cells is known to be required for spermatogenesis and male fertility, it remains poorly understood how autophagy participates in spermatogenesis. We found that systemic

knockout mice of Rubicon, a negative regulator of autophagy, exhibited a substantial reduction in testicular weight, spermatogenesis, and male fertility, associated with upregulation of autophagy. Rubicon-null mice also had lower levels of mRNAs of Sertoli cell-related genes in testis. Importantly, Rubicon knockout in Sertoli cells, but not in germ cells, caused a defect in spermatogenesis and germline stem cell maintenance in mice, indicating a critical role of Rubicon in Sertoli cells. In mechanistic terms, genetic loss of Rubicon promoted autophagic degradation of GATA4, a transcription factor that is essential for Sertoli cell function. Furthermore, androgen antagonists caused a significant decrease in the levels of Rubicon and GATA4 in testis, accompanied by elevated autophagy. Collectively, we propose that Rubicon promotes Sertoli cell function by preventing autophagic degradation of GATA4, and that this mechanism could be regulated by androgens.

2. FAM71F1 binds to RAB2A and RAB2B and is essential for acrosome formation and male fertility in mice

Akane Morohoshi [13, 14], Haruhiko Miyata [13], Yuki Oyama [13,15], Seiya Oura [13, 15], Taichi Noda [13, 16, 17], Masahito Ikawa [13,14,15,18]

[13]Department of Experimental Genome Research, Research Institute for Microbial Diseases, Osaka University.[14]Graduate School of Medicine, Osaka University.[15]Graduate School of Pharmaceutical Sciences, Osaka University.[16]Division of Reproductive Biology, Institute of Resource Development and Analysis, Kumamoto University.

[17]Priority Organization for Innovation and Excellence, Kumamoto University.

[18]Center for Infectious Disease Education and Research (CiDER), Osaka University.

The acrosome is a cap-shaped, Golgi-derived membranous organelle that is located over the anterior of the sperm nucleus and highly conserved throughout evolution. Although morphological changes during acrosome biogenesis in spermatogenesis have been well described, the molecular mechanism underlying this process is still largely unknown. Family with sequence similarity 71, member F1 and F2 (FAM71F1 and FAM71F2) are testis-enriched proteins that contain a RAB2B-binding domain, a small GTPase involved in vesicle transport and membrane trafficking. Here, by generating mutant mice for each gene, we found that Fam71f1 is essential for male fertility. In Fam71f1-mutant mice, the acrosome was abnormally expanded at the round spermatid stage, likely because of enhanced vesicle trafficking. Mass spectrometry analysis after immunoprecipitation indicated that, in testes, FAM71F1 binds not only RAB2B, but also RAB2A. Further study suggested that FAM71F1 binds to the GTP-bound active form of RAB2A/B, but not the inactive form. These results indicate that a complex of FAM71F1 and active RAB2A/B suppresses excessive vesicle trafficking during acrosome formation.

3. SPATA33 localizes calcineurin to the mitochondria and regulates sperm motility in mice

Haruhiko Miyata [4], Seiya Oura [4, 15], Akane Morohoshi [4, 14], Keisuke Shimada [4], Daisuke Mashiko [4], Yuki Oyama [4,15], Yuki Kaneda [4, 15], Takafumi Matsumura [4], Ferheen Abbasi [4], Masahito Ikawa [4, 15, 14]

Calcineurin is a calcium-dependent phosphatase that plays roles in a variety of biological processes including immune responses. In spermatozoa, there is a testis-enriched calcineurin composed of PPP3CC and PPP3R2 (sperm calcineurin) that is essential for sperm motility and male fertility. Because sperm calcineurin has been proposed as a target for reversible male contraceptives, identifying proteins that interact with sperm calcineurin widens the choice for developing specific inhibitors. Here, by screening the calcineurin-interacting PxIxIT consensus motif in silico and analyzing the function of candidate proteins through the generation of gene-modified mice, we discovered that SPATA33 interacts with sperm calcineurin via a PQIIIT sequence. *Spata33* knockout mice exhibit reduced sperm motility because of an inflexible midpiece, leading to impaired male fertility, which phenocopies *Ppp3cc* and *Ppp3r2* knockout mice. Further analysis reveals that sperm calcineurin disappears from the mitochondria in the *Spata33* knockout testis. In addition, immunoprecipitation analysis indicates that sperm calcineurin interacts with not only SPATA33 but also the mitochondrial protein VDAC2. These results indicate that SPATA33 localizes calcineurin to the mitochondria and regulates sperm motility.

4. ARMC12 regulates spatiotemporal mitochondrial dynamics during spermiogenesis and is required for male fertility

Keisuke Shimada [4], Soojin Park [4, 14], Haruhiko Miyata [4], Zhifeng Yu [16, 17], Akane Morohoshi [4, 14], Seiya Oura [4,15], Martin M Matzuk [16,17], Masahito Ikawa [4,14,15]

[16]Center for Drug Discovery, Baylor College of Medicine.[17]Department of Pathology & Immunology, Baylor College of Medicine.

The mammalian sperm midpiece has a unique double-helical structure called the mitochondrial sheath that wraps tightly around the axoneme. Despite the remarkable organization of the mitochondrial sheath, the molecular mechanisms involved in mitochondrial sheath formation are unclear. In the process of screening testis-enriched genes for func-

tions in mice, we identified armadillo repeat-containing 12 (ARMC12) as an essential protein for mitochondrial sheath formation. Here, we engineered *Armc12*-null mice, FLAG-tagged *Armc12* knock-in mice, and TBC1 domain family member 21 (*Tbc1d21*)-null mice to define the functions of ARMC12 in mitochondrial sheath formation in vivo. We discovered that absence of ARMC12 causes abnormal mitochondrial coiling along the flagellum, resulting in reduced sperm motility and male sterility. During spermiogenesis, sperm mitochondria in *Armc12*-null mice cannot elongate properly at the mitochondrial interlocking step which disrupts abnormal mitochondrial coiling. ARMC12 is a mitochondrial peripheral membrane protein and functions as an adherence factor between mitochondria in cultured cells. ARMC12 in testicular germ cells interacts with mitochondrial proteins MIC60, VDAC2, and VDAC3 as well as TBC1D21 and GK2, which are required for mitochondrial sheath formation. We also observed that TBC1D21 is essential for the interaction between ARMC12 and VDAC proteins in vivo. These results indicate that ARMC12 uses integral mitochondrial membrane proteins VDAC2 and VDAC3 as scaffolds to link mitochondria and works cooperatively with TBC1D21. Thus, our studies have revealed that ARMC12 regulates spatiotemporal mitochondrial dynamics to form the mitochondrial sheath through cooperative interactions with several proteins on the sperm mitochondrial surface.

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Center for Experimental Medicine and Systems Biology

Division of Genome Engineering
ゲノム編集研究分野

Professor	Tomoji Mashimo, Ph.D.	教　授	博士(人間・環境学)	真　下　知　士
Senior Assistant Professor	Kazuto Yoshimi, Ph.D.	講　師	博士(医科学)	吉　見　一　人

Genome engineering technologies, such as Zinc finger nucleases (ZFNs), TAL effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR)-associated (Cas) nucleases (CRISPR-Cas), have widely used in life and medical science. We have developed a novel genome editing tool, CRISPR-Cas3 to overcome technical and patent limitation of CRISPR-Cas9 system. We are analyzing molecular mechanisms underlying Cas3-mediated genome editing in human cells and also developing some efficient genome editing strategies with these tools in rodents. These technologies facilitate easy and flexible gene editing in living organisms.

Dynamic mechanisms of CRISPR interference by Escherichia coli CRISPR-Cas3

Kazuto Yoshimi, Kohei Takeshita[1], Noriyuki Kodera[2], Satomi Shibumura, Yoko Yamauchi, Hiromi Taniguchi, Tomoaki Fujii, Mine Omatsu1, Yayoi Kunihiro, Masaki Yamamoto[1], Tomoji Mashimo
1 , Life Science Research Infrastructure Group,Advanced Photon Technology Division, RIKEN SPring-8 Center, Hyogo 679-5148 Japan
2, Nano Life Science Institute (WPI-NanoLSI), Kanazawa University, Kakuma-machi, Kanazawa, 920-1192, Japan

Type I CRISPR-Cas3 uses an RNA-guided multi Cas-protein complex, Cascade, which detects and degrades foreign nucleic acids via the helicase-nuclease Cas3 protein. Despite many studies using cryoEM and smFRET, the precise mechanism of Cas3-mediated cleavage and degradation of target DNA remains elusive. Here we reconstitute the CRISPR-Cas3 system in vitro to show how the Escherichia coli Cas3 (EcoCas3) with EcoCascade exhibits collateral non-specific ssDNA cleavage and target specific DNA degradation. Partial binding of EcoCascade to target DNA with tolerated mismatches within the spacer sequence, but not the PAM, elicits collateral ssDNA cleavage activity of recruited EcoCas3. Conversely, stable binding with complete R-loop formation drives EcoCas3 to nick the non-target strand (NTS) in the bound DNA. Helicase-dependent unwinding then combines with trans ssDNA cleavage of the target strand and repetitive cis cleavage of the NTS to degrade the target dsDNA substrate. High-speed atomic force microscopy demonstrates that EcoCas3 bound to EcoCascade repeatedly reels and releases the target DNA, followed by target fragmentation. Together, these results provide a revised model for collateral ssDNA cleavage and target dsDNA degradation by CRISPR-Cas3, furthering understanding of type I CRISPR priming and interference and informing future genome editing tools.

CRISPR-Cas3-based diagnostics for SARS-CoV-2 and influenza virus

Kazuto Yoshimi, Kohei Takeshita[1], Seiya Yamayoshi[2], Satomi Shibumura[3], Yuko Yamauchi, Masaki Yamamoto[1], Hiroshi Yotsuyanagi[4],Yoshihiro Kawaoka[2,5], and Tomoji Mashimo
1, Life Science Research Infrastructure Group,Advanced Photon Technology Division, RIKEN SPring-8 Center, Hyogo 679-5148 Japan
2, Division of Virology, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, Minato-ku, 108-8639, Tokyo, Japan
3, C4U Corporation, Osaka 565-0871, Japan,
4, Division of Infectious Diseases and Applied Immunology, Institute of Medical Science, University

of Tokyo, Minato-ku, 108-8639,Tokyo, Japan,
5, Department of Pathobiological Sciences, School of Veterinary Medicine,
University of Wisconsin-Madison, Madison 53711, Wisconsin, USA

CRISPR-based diagnostics (CRISPR-dx), including the Cas12-based DETECTR and Cas13-based SHERLOCK Class 2 CRISPRs, have been used to detect the presence of DNA or RNA from pathogens, such as the 2009 pandemic influenza virus A (IVA) and the 2019 novel coronavirus SARS-CoV-2. Here, the collateral single-stranded DNA cleavage we observed with Class 1 type I CRISPR-Cas3 highlights its potential for development as a Cas3-mediated rapid (within 40 min), low-cost, instrument-free detection method for SARS-CoV-2. This assay, which we called Cas3-operated nucleic acid detection (CONAN), not only detects SARS-CoV-2 in clinical samples, but also offers the specific detection of single-base-pair mutations in IVA variants. This tool allows rapid and accurate point-of-care testing for patients with suspected SARS-CoV-2 or drug-resistant IVA infections in hospitals.

Generation of several genetically engineered rat models via Combi-CRISPR method

Kazuto Yoshimi, Yuichiro Oka[1,2], Yoshiki Miyasaka[3], Yuko Kotani[3], Misato Yasumura[1], Yoshihiro Uno[3], Kosuke Hattori[3], Arisa Tanigawa[3], Manami Oya[4], Kazuhiro Nakamura[4], Toshihide Yamashita[5], Makoto Sato[1,2], Natsuki Matsushita[6], Kazuto Kobayashi[7], and Tomoji Mashimo
1, Department of Anatomy and Neurosciences, Graduate School of Medicine, Osaka University, Osaka 565-0871, Japan
2, Department of Child Development, United Graduate School of Child Development, Osaka University, Osaka 565-0871, Japan
3, Institute of Experimental Animal Sciences, Graduate School of Medicine, Osaka University, Osaka 565-0871, Japan
4, Department of Integrative Physiology, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan
5, Department of Molecular Neuroscience, Graduate School of Medicine, Osaka University, Osaka 565-0871, Japan
6, Division of Laboratory Animal Research, Aichi Medical University School of Medicine, Aichi 480-1195, Japan
7, Department of Molecular Genetics, Institute of Biomedical Sciences, Fukushima Medical University School of Medicine, Fukushima 960-1295, Japan

CRISPR-Cas9 systems have been widely used for gene targeting in mice and rats. The non-homologous end joining (NHEJ) repair pathway, which is dominant in zygotes, efficiently induces insertion or deletion (indel) mutations as gene knockouts (KOs) at targeted sites, whereas gene knock-ins (KIs) via homology-directed repair (HDR) are difficult to generate. We have used a double-stranded DNA (dsDNA) donor template with Cas9 and two single guide RNAs (sgRNAs), one designed to cut the targeted genome sequences and the other to cut both the flanked genomic region and one homology arm of the dsDNA plasmid, resulting in 20%–50% KI efficiency among G0 pups. This combinational method of NHEJ and HDR mediated by the CRISPR-Cas9 system, named Combi-CRISPR, facilitates the efficient and precise KIs of plasmid DNA cassettes in mice and rats. We have established genetically-modified rats via Combi-CRISPR with several collaborators.

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Center for Experimental Medicine and Systems Biology

Core Laboratory for Developing Advanced Animal Models
先進モデル動物作製コア

Professor	Yasuhiro Yamada, M.D., Ph.D.	教　授	博士(医学)	山　田　泰　広
Professor	Tomoji Mashimo, Ph.D.	教　授	博士(人間・環境学)	真　下　知　士
Associate Professor	Manabu Ozawa, Ph.D	准教授	博士(農学)	小　沢　　学

The aim of the 'Core Laboratory for Developing Advanced Animal Models' is to support basic sciences in the life science field by producing and providing gene manipulated mice or rats such as human disease models or gene KO/KI models. Using cutting-edge genome editing techniques, we produce various types of gene manipulated animals including indel mutation, large fragment deletion, SNPs, conditional Cre/loxP, drug inducible gene expression/silencing, reporter gene KI, or gene conversion for making humanized mice or rat models either by direct gene editing in zygote or highly efficient ES cell-mediated gene targeting followed by chimera animal productions.
https://www.ims.u-tokyo.ac.jp/cemsb/public_html/index.html

Laboratories that consist the Core

'Core Laboratory for Developing Advanced Animal models' is launched in 2020 for the purpose of providing gene manipulated mice or rats model to domestic or international academic institutions. Two divisions, Division or Stem Cell Pathology and Division of Genome engineering, and one laboratory, Laboratory of Reproductive Systems Biology, of which all belong to Center for Experimental Medicine and Systems Biology consists the Core.

Cutting edge genome editing techniques

For making indel mutants, large deletion, or short DNA fragment KI such as SNPs, or peptide tags, we offer direct genome editing using mouse or rat zygote through NEPA electroporation systems (NEPA Gene). In mice, embryos from C57BL/6J strain are routinely served for genome editing, but other strains such as C57BL/6N or BDF1 are also applicable if necessary. In the rat, F344/Jcl strain is served for zygote genome editing. For large size gene manipulations in mice, such as Cre/loxP conditional allele, fluorescein reporters KI, gene conversion from mice to human, or drug-inducible Tet-on/off system, we offer CRISPR/Cas9-assisted plasmid KI using ES cells through Neon Electroporation system (ThermoFisher) followed by blastocyst injection for developing chimeric mice. ES cells from C57BL/6J, C57BL/6N, 129, B6129F1, or Balb/c strains are available for chimera productions. For producing large size gene manipulated rat, e.g., reporter KI or humanized rat models, direct zygote genome editing technique, terms Combi-CRISPR, is applicable.

Supporting gene manipulated mouse or rat model production through the core lab and AdAMS platform

We provide our cutting-edge animal production techniques through our core lab as well as Advanced Animal Model Support, AdAMS. Our core is a member of AdAMS, which belongs to the Committee on Promoting Collaboration in Life Science, MEXT, and is an academic platform for producing gene manipulated animals. Any researchers who have KAKENHI, Grant-in-Aid for Scientific Research, are eligible for

applying to this platform.

Number of mice or rat strains we have developed in 2020

In 2021, our core has provided 11 or 11 strains of gene manipulated mice through the core lab or AdAMS, respectively. In the rat case, 2 or 6 strains of gene manipulated rats have also been provided through the core lab or AdAMS, respectively.

Advanced Clinical Research Center

Division of Cellular Therapy
細胞療法分野

Professor	Toshio Kitamura, M.D., D.M.Sc.	教　授	医学博士	北　村　俊　雄
Assistant Professor	Tomofusa Fukuyama, M.D., D.M.Sc.	助　教	博士(医学)	福　山　朋　房
Assistant Professor	Yosuke Tanaka, Ph.D.	助　教	博士(医学)	田　中　洋　介
Assistant Professor	Yutaka Enomoto, D.M.Sc.	助　教	博士(医学)	榎　本　　豊

Our major projects are (1) Co-ordinate control of cell division and differentiation by a crosstalk between JAK/STAT and small GTPases, (2) Molecular targeted therapies, and (3) Elucidation of molecular basis of leukemia, hematological malignancioes.

1. Co-ordinate control of cell division and cell differentiation of by the Rho family small GTPases.

Takeshi Fukushima, Yosuke Tanaka, Toshihiko Oki, Toshiyuki Kawashima, Kohtaro Nishimura, Susumu Goyama, and Toshio Kitamura.

In search for key molecules that prevent murine M1 leukemic cells from undergoing IL-6-induced differentiation into macrophages, we previously isolated an antisense cDNA that encodes full-length mouse MgcRacGAP through functional cloning. In human HL-60 leukemic cells, overexpression of human MgcRacGAP induced differentiation to macrophages. Interestingly, MgcRacGAP localized to the nucleus in interphase, accumulated to the mitotic spindle in metaphase, and was condensed in the midbody during cytokinesis. The GAP activity of MgcRacGAP was required for completion of cytokinesis. We also found that MgcRacGAP is phosphorylated by Aurora B at the midbody. Intriguingly, this phosphorylation induced the Rho-GAP activity of MgcRacGAP, which was critical for completion of cytokinesis. We identified S387 as a phosphorylation site responsible for the acquirement of Rho-GAP activity during cytokinesis at the midbody. On the other hand, MgcRacGAP mainly localizes in the nucleus in the interphase. We demonstrated that MgcRacGAP directly bound transcription factors STAT3 and STAT5, and enhanced transcriptional activation of STAT proteins as a Rac GAP. MgcRacGAP was found to harbor functional NLS and works as a nuclear chaperon together with Rac1.

We also found using an MgcRacGAP-GFP fusion protein that MgcRacGAP expression increased in the early G1 phase in parallel with or even earlier than Geminin, suggesting that MgcRacGAP may play roles in G1 check point. MgcRacGAP accumulates to the midbody during cytokinesis, and the midbody is included in one of the daughter cells after cell division. It was suggested by some researchers that the midbody is frequently released from the cells in stem cells. We therefore hypothesized that the cells with midbody tend to differentiate and the cells without midbody tend to self-renew or enter G0 phase. To test this hypothesis, we have recently generated a transgenic mouse expressing the MgcRacGAP-mVenus fusion protein in hematopoietic stem cells and/or progenitors.

2. Molecular basis of acute leukemia, myelodysplastic syndromes (MDS), MDS overt leukemia, and myeloproliferative neoplasms (MPN).

Daichi Inoue, Reina Nagase, Takeshi Fujino, Yasutaka Hayashi, Shuji Asada, Reina Takeda, Kojin C Kawabata, Naoko Watanabe, Makoto Saika, Yukiko Komeno, Naoko Kato, Yutaka Enomoto, Toshihiko Oki, Yuka Harada[1], Hironori Harada[2], Tetsuya Nosaka[3], Jiro Kitaura[4], Yosuke Tanaka, Tomofusa Fukuyama, Susumu, Goyama, and Toshio Kitamura: [1]Department of Clinical Laboratory Medicine, Bunkyo Gakuin University, Laboratory of Oncology, [2]Department of Medical Science Tokyo University of Pharmacy and Life Sciences, [3]Mie University School of Medicine, and [4]Allergy Center, Juntendo University.

Recent progress using high-speed sequencing has identified mutations in genes that are not categorized to class I and class II mutations, including epigenetic factors, and splicing factors. We established two MDS models induced by ASXL1 mutations and EZH2 mutations; mice transplanted with bone marrow cells expressing C-terminal truncating mutants of ASXL1 derived from MDS patients or a catalytic domain (SET)-deleted mutant of EZH2 (EZH2-dSET) developed MDS-like diseases in a year or two. Concerning the molecular mechanisms, the ASXL1 mutant (ASXL1-MT) suppressed PRC2 and MLL fuctions, leading to the derepression of posterior HoxA genes and miR125a via inhibition of H3K27me3 and decreased expression of Id2 and TJP1 via inhibition of HeK4me3. In addition, ASXL1-MT stabilizes and activate BAP1, leading to the derepression of IRF8 and Bcl2 via decreased H2AK119Ub. Thus, ASXL1-MT changes cellular programs via reduced H3K4me3, H3K27me3 and H2AK119Ub. ASXL1 mutations are frequently associated with SETBP1 mutations (SETBP1-MT) that stabilize SETBP1 and SET oncoprotein, leading to activation of the PI3K/Akt pathway. In the BMT model, combination of ASXL1-MT and SETBP1-MT induced AML with much shorter latencies. GSEA indicated that the TGF beta pathway was profoundly inhibited, implying the inhibition of the TGF beta pathway in leukemic transformation of MDS. Further experiment is now under way to clarify the molecular mechanisms by which the TGF beta pathway was inhibited.

3. Clonal hematopoiesis and hematological malignancies.

Takeshi Fujino, Daichi Inoue, Reina Takeda, Shuhei Asada, Yuka Hayashi, Tomofusa Fukuyama, Yosuke Tanaka, Susumu Goyama, Toshio Kitamura

Recent progress in deep sequencing technologies revealed the presence of blood clone harboring one or two leukemia-related mutations in 10% of apparently healthy individuals older than 65-years old. The mutated genes include epigenetic facors, splicing factors, cohesin-complex genes, signaling molecuels and p53. The most frequent mutations are found in DNMT3a followed by TET2 and ASXL1 which represent 60~70% of CH. People with CH have 10-times higher risk for developing hematological malignancies. In addition, they have 2-times higher risks for developing stroke and acute myocardial infarction. Interestingly, in cancer patients after chemotherapy, CH is identified in 20~30% patients. Importantly, cancer patients with clonal hematopoiesis have higher ratios of recurrence and poorer prognosis. Now we are characterizing ASXL1-MT-KI mice to clarify how people with clonal hematopoiesis develop a variety of diseases.

Recently established Rosa26-knock-in mice for ASXL1-MT do not develop MDS, but presented disturbed differentiation of erythroid cells and mild macrocytic anemia. Combination with other mutations (eg. Runx1mutation) and insertional mutagenesis experiments have demonstrated that these mice are in the pre-leukemic states. Now we use the ASXL1-MT-KI mouse as a model for "clonal hematopoiesis".

3. Investigating molecular pathogenesis of AML1-MTG8/ETO leukemia

Tomofusa Fukuyama, Susumu Goyama, Toshio Kitamura

t(8;21) AML is the most common cytogenetic subtype of AML, and the resultant AML1-MTG8 chimeric protein is believed to play an important role for leukemogenesis. However, the role of AML1-MTG8 is still unclear because persistent existence of chimeric gene including chimeric fusion point detected by PCR is observed in complete remission or healthy persons, even in utero. In addition, full length of AML1-MTG8 by itself cannot cause leukemia in mouse models, suggesting that additional "events" should be required for leukemogenesis. We have identified a new splicing variant that has significant ability to induce leukemia in mouse model. Mechanisms of leukemogenesis by it as well as clinical significance are currently under investigation.

4. Development of RUNX1-targeted therapy for AML

Kohei Iida, Taishi Yonezawa, Tomofusa Fukuyama, Yosuke Tanaka, Hirotaka Takahashi[5], Tatsuya Sawasaki[5], Toshio Kitamura, Susumu Goyama
[5]Proteo-Science Center (PROS), Ehime University

RUNX1 is a transcription factor and plays important roles in hematopoiesis. Recent reports have

shown that RUNX1 promotes the development of leukemias as well as several solid cancers. CBFB is a partner protein of RUNX1 composing heterodimer, and it increases the stability and DNA-binding ability of RUNX1. Therefore, inhibition of RUNX1-CBFB interaction has been considered a promising therapeutic strategy for RUNX1-dependent tumors. However, the effects of RUNX1-CBFB inhibition have not been proven in clinical trials.

To develop novel small molecule inhibitors of RUNX1-CBFB interaction, we developed a luminescence-based interaction assay (AlphaScreen) to quantify the RUNX1–CBFB interaction using recombinant proteins synthesized with the wheat cell-free system. We screened a core library collection of 9,600 compounds (provided from Drug Discovery Initiative of Tokyo University) with the AlphaScreen, and identified several candidate compounds. We then assessed the effect of these candidate compounds in a cell-based assay, and found that two compounds indeed inhibit the RUNX1-CBFB interaction. We also confirmed the growth-inhibitory effect of the compounds on Jurkat cells and TF-1 cells, whose growth was shown to be suppressed by genetic depletion of RUNX1. The compounds identified in this study are promising lead compounds to treat RUNX1-dependent leukemias and cancers.

In addition to the RUNX1-CBFB interaction inhibitor, we are developing proteolysis-targeting chimeric molecules (PROTACs) to induce RUNX1 degradation. Using the above mentioned AlphaScreen, we identified STUB1 as an E3 ligase to promote RUNX1 ubiquitination and degradation. We also identified several compounds that bind to RUNX1 and STUB1. These finding will be the basis to develop RUNX1-STUB1 PROTACs in future.

Furthermore, we examined the effect of lipid nanoparticle (LNP)-mediated delivery of RUNX1-targeting siRNA on the growth of leukemia cells. The tumor-tropic LNPs containing RUNX1-targeting siRNA were incorporated into myeloid and T-cell leukemia cell lines and the patient-derived primary human AML cells, downregulated RUNX1 expression, induced cell cycle arrest and apoptosis, and showed the growth-inhibitory effect in them. Importantly, the LNPs were not efficiently incorporated into normal cord blood CD34+ cells, showing minimum cytotoxicity in them. These finding suggest that the LNP-based siRNA delivery as a promising approach to deplete RUNX1 specifically in leukemia cells.

5. Targeting TP53 and antitumor immunity for leukemia therapy

Emi Sugimoto, Yasutaka Hayashi, Susumu Goyama, XiaoXiao Liu, Moe Tamura, Shuhei Asada, Yosuke Tanaka, Tomofusa Fukuyama, Toshio Kitamura

The negative regulator of p53, MDM2, is frequently overexpressed in acute myeloid leukemia (AML) that retains wild-type *TP53* alleles. Targeting of p53-MDM2 interaction to reactivate p53 function is therefore an attractive therapeutic approach for AML. We showed that an orally active inhibitor of p53-MDM2 interaction, DS-5272, causes drastic tumor regressions of MLL-AF9-driven AML *in vivo* with a tolerable toxicity. However, the antileukemia effect of DS-5272 was markedly attenuated in immunodeficient mice, indicating the critical impact of systemic immune responses that drive p53-mediated leukemia suppression. In relation to this, DS-5272 triggered immune-inflammatory responses in MLL-AF9 cells including upregulation of Hif1α and PD-L1, and inhibition of the Hif1a-PD-L1 axis sensitized AML cells to p53 activation.

To determine the role of specific immune cells in the development of AML, we compared the latencies of MLL-AF9-induced AML in immunologically normal wild-type (WT) C57BL/6 mice, $Rag2^{-/-}$ mice lacking mature T and B cells, and NSG mice lacking T, B and NK cells. As expected, we observed earlier onset of AML in the immunodeficient NSG mice. To our surprise, AML progression was significantly retarded in $Rag2^{-/-}$ mice even compared to WT mice with normal immune function. Furthermore, the survival advantage of $Rag2^{-/-}$ mice became more evident when the mice were treated with a p53 activating drug DS-5272. Intriguingly, NK cells in $Rag2^{-/-}$ mice were increased in number, highly expressed activation markers, and showed increased cytotoxicity to leukemia cells in the coculture assay. B2m depletion in MLL-AF9 cells resulted in impaired AML progression, while NK cell depletion in $Rag2^{-/-}$ mice accelerated it. These data suggest that NK cells play a central role in suppressing the development of MLL-AF9-driven AML. Our study also revealed that $Rag2^{-/-}$ mice, which are generally considered as "immunodeficient" due to the lack of functional lymphocytes, in fact have hyperactive NK cells.

Taken together, our findings suggests that dual activation p53 and NK cells could be a promising therapeutic strategy for AML.

6. Development of novel combination therapies with TKIs for CML-LSCs

Yosuke Tanaka, Tsuyoshi Fukushima, Susumu Goyama, Toshio Kitamura

CML LSCs (Chronic myeloid leukemia leukemic stem cells) were thought to be quiescent (in G_0 phase). Their quiescent state is thought to be a major reason why CML LSCs are resistant to several BCR-ABL tyrosine kinase inhibitors (TKIs). We planned to examine relationships between quiescent states of CML LSCs, their leukemia-initiating potential and their resistance to TKIs. To this end, we have established mouse CML model with G_0M. Briefly, we retrovirally

overexpressed BCR-ABL fusion gene in bone marrow (BM) cells from 5FU-treated G_0M mice and then injected them into lethally irradiated wild type mice to develop CML mouse model. These mice developed CML within 3 weeks. We found that G_0M and CD27, a marker for CML stem and progenitor cells, could split the conventional CML LSC fraction (BCR-ABL+, cKit+, Sca1+, lineage marker negative; CML KSL), into 4 fractions and identified that CML LSCs and CML progenitor cells were enriched in G_0M-postive CD27-postive CML KSL fraction and G_0M-negative CD27-postive CML KSL fraction, respectively. We confirmed that the CML LSCs were resistant to imatinib. RNA-Seq analysis exhibited that NFkB signaling pathways were enriched in imatinib-treated CML LSCs as compared with vehicle-treated CML LSCs, indicating that NFkB signal pathways are important for their resistance to imatinib. To inhibit NFkB signal pathways, we used IRAK1/4 inhibitor, which can block IL1R/TLR4 signals to activate NFkB. Combination therapy of imatinib with IRAK1/4 inhibitor was effective at eliminating CML LSCs as compared with mono therapies. We extended the examination of efficacy of IRAK1/4 inhibitor on CML LSCs to CML-CP patient samples. IRAK1/4 inhibitor in combination with imatinib was effective at eliminating CD34+CD38-CD90+ CML LSCs in vitro and xenograft model. These results exhibited that IRAK1/4-NFkB signal axis was crucial for maintenance of imatinib resistant CML LSCs.

Moreover, we found that the CML LSCs expressed PD-L1 at higher level than the CML progenitor cells and normal HSCs (Hematopoietic stem cells). PD-L1 expression is known to be regulated by NFkB. Actually, inhibition of NFkB activation by IRAK1/4 inhibitor attenuated PD-L1 expression on CML LSCs, indicating that the efficacy of IRAK1/4 inhibitor on eradication of CML LSCs could be partially due to the attenuation of PD-L1 expression on CML LSCs. To assess this possibility, we performed the combination therapy of imatinib and PD-L1 blocking antibody for eradication of CML LSCs. The combination was more effective at eliminating CML LSCs than mono therapies. We also observed that the combination efficacy was abolished in the absence of T cell immunity.

IRAK1/4 inhibitor in combination with imatinib eradicated CML LSCs in the absence of T cell immunity, indicating that IRAL1/4 inhibitor directly eradicates CML LSCs probably by its proapoptotic function. Moreover, IRAK1/4 inhibitor attenuated PD-L1 expression on CML LSCs and anti-PD-L1 antibody with imatinib also eradicated CML LSCs in the presence of T cell immunity. In summary, the IRAK1/4 inhibitor in combination with imatinib eradicated CML LSCs by ducal functions. One is its proapoptotic function and the other is induction of anti-tumor immunity by the attenuation of immune check point molecules. Thereby, IRAK1/4 inhibitor is an attractive and powerful drug in eradicating CML LSCs with TKIs.

7. Clonal hematopoiesis and various disorders including hematological malignancies, cardiovascular diseases, and carcinomas.

Takeshi Fujino, Reina Takeda, Shuhei Asada, Keiichi Yamamoto, Liu Xiaoxiao, Naru Sato, Tomohiro Yabushita, Yuka Hayashi, Tomofusa Fukuyama, Yosuke Tanaka, Daichi Inoue, Susumu Goyama, Toshio Kitamura

Recent progress in deep sequencing technologies revealed the presence of blood clone harboring one or two leukemia-related mutations in 10% of apparently healthy individuals older than 65-years old. The mutated genes include epigenetic facors, splicing factors, cohesin-complex genes, signaling molecuels and p53. TSUTAYA most frequent mutations are found in DNMT3a followed by TET2 and ASXL1. This is called clonal hemopopoiesis (CH or CHIP) and people with CH develop hematological malignancies at 10-fold frequencies. In addition to hematological malignancies, peolpe with CH show 2-fold higher risk to get cardiovascular diseases including myocardial infarction and cerebral infarction. To investigate the etiologies, we utilized the ASXL1-MT-KI mouse as a model for CH. We found that ASXL1-MT activates Akt by deubiquitinating phosphorylated Akt in collaboration with BAP1. This is an interesting finding showing a novel role of ASXL1 in the cytoplasm given that ASXL1 is an epigenetic factor and was thought to work in the nucleus. We also found that a transcription factor HHEX collaborates with ASXL1-MT in enhancing leukemogenesis. In addition, we have recently found that ASXL1 is a critical component of nuclear paraspeckle and that ASXL1-MT disrupts the nuclear paraspeckle, leading to the reduced hematopoietic stem cell functions. Thus, ASXL1 has a variety of functions both in the nucleus and the cytoplasm and disruption of ASXL1 by mutations could induce a variety of diseases. In relation to this, we are now conducting experiments to investigate the relationship between CH and CVDs or carcinomas.

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Advanced Clinical Research Center

Division of Infectious Diseases
感染症分野

Professor	Hiroshi Yotsuyanagi, M.D., D.M.Sc.	教　授	博士(医学)	四　柳	宏
Associate Professor	Takeya Tsutsumi, M.D., D.M.Sc.	准教授	博士(医学)	堤	武也
Project Assistant Professor	Michiko Koga, M.D., D.M.Sc.	特任助教	博士(医学)	古　賀	道子
Assistant Professor	Makoto Saito, M.D., DPhil.	助　教	博士(医学)	齋　藤	真

Our overall goal is medical sciences on infectious diseases in two directions, from clinic to bench and from bench to clinic. Our main subjects have been immunopathogenesis of HIV-1 infection in addition to other viruses, especially hepatitis viruses. Since the emergence of SARS-CoV2, we started the basic and clinical research using clinical samples obtained from SARS-CoV2-infected patients admitted to the IMSUT Hospital, in order to settle down COVID-19. (68/70 words)

1. Clinical research of COVID-19

Makoto Saito, Eisuke Adachi[1], Hiroyuki Nagai[1], Kazuhiko Ikeuchi, Michiko Koga, Takeya Tsutsumi, Hiroshi Yotsuyanagi

[1] Department of Infectious Diseases and Applied Immunology, IMSUT Hospital

Since the emergence and spread of SARS-CoV2 in Japan in the beginning of 2020, many patients with COVID-19 were admitted to the IMSUT Hospital. There are more than 500 COVID-19 patients admitted to our hospital in 2020-2021. By the observation of these patients, we noticed a lot of clinical questions and investigated various factors such as patients' backgrounds, clinical findings, and laboratory data. We have obtained many novel and interesting findings and published them in international journals. The medical care for COVID-19 patients is ongoing at the IMSUT Hospital and the number of patients is accumulating, therefore we continue the investigation which will contribute to the improvement of patient care and understanding of COVID-19.

2. Basic research for the control of COVID-19

Michiko Koga, Makoto Saito, Eisuke Adachi[1], Hiroyuki Nagai[1], Shinya Yamamoto, Kazuhiko Ikeuchi, Takeya Tsutsumi, Aya Ishizaka, Taketoshi Mizutani[2], Ai Tachikawa-Kawana[3], Ken Ishii[4], Yoshihiro Kawaoka[5], Hiroshi Yotsuyanagi

[2] Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo

[3] AIDS Research Center, National Institute of Infectious Diseases

[4] Division of Vaccine Science, IMSUT

[5] Division of Virology, IMSUT

Several laboratories at IMSUT and external institutes have continued COVID-19-related research during 2021, and we have been working in collaboration with some of these laboratories. Our main mission is to obtain and share clinical samples from COVID-19 patients, but we are also conducting basic research by ourselves such as microbiomes in those patients. Additionally, we are attempting to perform high-resolution transcriptomic analysis of blood immune cells from disease progression to recovery in COVID-19 in order to enhance a better understanding of the protective and pathogenic immune responses of the disease. Specifically, we are performing single-cell RNA se-

quencing (scRNA-seq) to obtain a bias-free and comprehensive imaging of immune responses in peripheral blood mononuclear cells (PBMCs) from patients with COVID-19.

3. Analysis of genetic sequence of hepatitis viruses.

Takeya Tsutsumi, Kazuaki Takahashi, Kazuhiko Ikeuchi, Eisuke Adachi[1], Michiko Koga, Hiroshi Yotsuyanagi

We sometimes see patients with acute hepatitis at IMSUT Hospital. Most of the causes are viral hepatitis, induced by hepatitis A, B, C, or E virus. Actually, in 2018, there was the outbreak of hepatitis A in HIV-infected patients, and until 2021 we sometimes see some patients with hepatitis A. Concerning hepatitis B and C, due to the similar route of infection, every year we see some patients who are also infected with HIV. Using sera and/or stools obtained from these patients, we cloned a total or part of viral genome and determined the genetic sequence of the viruses, to identify the transmission route of the viruses and also the drug-resistant mutations or vaccine escape mutations. Concerning hepatitis B, we have also been examining the HBV-positive samples derived from blood donors who were accidentally found to be HBsAg-positive. As for hepatitis E, we are collaborating with outside researchers in Tokyo as well as Hokkaido where the hepatitis E virus infection is sometimes observed. By cloning the virus from samples derived from not only the patients but also suspectable foods or wild animals, we try to investigate the transmission routes of the virus.

4. Evaluation of the efficacy of HA vaccine for HIV-MSM and analysis on NAFLD in HIV infected patients

Michiko Koga, Takeya Tsutsumi, Kazuhiko Ikeuchi, Lay Ahyoung Lim[1],Tadashi Kikuchi[1], Eisuke Adachi[1], Hiroshi Yotsuyanagi

Due to the outbreak of hepatitis A in Japan around 2018, we started to vaccinate HIV-MSM with HA vaccine "Aimmugen®" in IMSUT Hospital. Aimmugen® is shown to be highly effective and induce IgG-HA antibodies for almost all of healthy people by twice vaccination. However, there have been the efficacy for HIV-infected people, especially in case of twice vaccination. Therefore, we evaluated the efficacy of Aimmugen® among HIV-MSM, particularly focused on twice vaccination. By October 2019, 147 HIV-MSM were vaccinated at least once with Aimmugen®. Among them, 134 finished the second vaccination and 114 were tested for IgG-HA antibodies. Ninety-five HIV-MSM were seropositive for IgG-HA, indicating the seropositive rate after second vaccination is 71.1%, which is lower than healthy adults. In 114 subjects whose anti-HA-IgG titers were tested after the second dose, factors significantly associated with better response were prolonged ART duration and higher CD4 count. The titers of anti-HA-IgG after the third dose were higher in those who became seropositive after the second dose than those who did not.

Now, we have started to evaluate NAFLD of HIV infected patients. Of the 102 HIV-infected patients, the prevalence of NAFLD was estimated to be 53.9% from the elastography CAP value, and 7.8% were suspected of progressing fibrosis and immediate improvement in metabolic risk factors is desired.

5. Characteristics of Transmitted Drug-Resistant HIV-1 in Recently Infected Treatment-Naive Patients in Japan.

Michiko Koga, Kazuhiko Ikeuchi, Eisuke Adachi[1], Tadashi Kikuchi[1], Takeya Tsutsumi, Hiroshi Yotsuyanagi

Progress in antiretroviral treatment has led to fewer virological failure cases, but about 10% of treatment-naive HIV/AIDS cases are reported to harbor drug-resistant strains, suggesting transmission of drug-resistant HIV. We have determined the trend in prevalence of transmitted drug-resistant (TDR) HIV in Japan from 2003.

Drug-resistance test had been performed on national-wide HIV-1-infected cases newly diagnosed. The overall prevalence of TDR was about 9.2% in 2020.

6. Exploratory research of the malignancy with HIV infected hemophilia patients

Michiko Koga, Takahiro Tanaka[1], Aya Ishizaka, Takashi Hosaka[1], Hiroshi Yotsuyanagi,

It is speculated that hemophiliacs infected with HIV due to chemical damage are more likely to get malignancy due to aging and immune dysfunction. Since April 2021, we have started this research with the following four objectives. 1. Construction and operation of a system design for a health examination. 2. Medical support at the time of diagnosis of malignancy and after diagnosis. 3. Mental care associated with malignancy. 4. Public relations regarding support and diseases.

We also reported that HIV-infected patients suffered much stress under the COVID-19 pandemic.

7. Identification of drugs which reactivate latent HIV-1 reservoir

Ayako Sedohara, Michiko Koga, Makoto Saito, Kazuhiko Ikeuchi, Eisuke Adachi[1], Tomohiko Koibuchi[1], Hiroshi Yotsuyanagi

The eradication of HIV provirus from HIV-infected patients is a crucial for curing HIV. A population of HIV-infected CD4 T-cells represents a latent infection, or reservoir. The latently infected cells can be reactivated by treatment with an latency reversing agent (LRA), allowing eradication by the host immune system, also termed the kick-and-kill strategy. It has been reported that histone acetyltransferase inhibitor SAHA strongly reactivates latent HIV reservoir. Using CD4 T-cells derived from HIV-infected individuals, we tried to identify novel chemical compounds that act as an LRA. Consequently, we identified that valemetostat/DS-3201/(*R*)-OR-S2, novel enhancer of zeste homolog 1/2 (EZH 1/2) dual inhibitor, acts as an LRA. EZH2 is a component of polycomb repressive complex 2 (PRC2) and functions as a methyltransferase. EZH2 methylates lysine 27 on histone H3 protein (H3K27). Tri-methylated H3K27 (H3K27me3) marks the gene silencing region and is mainly observed around the 5'LTR, the transcriptional regulatory region, in latently infected HIV-1 cells. EZH2 is involved in the maintenance of HIV-1 latency. Treatment of HIV-1 latently infected cells with valemetostat reversed latency *in vitro* and *ex vivo* in a dose- and duration-dependent manner at levels similar to SAHA. Furthermore, co-administration of SAHA with valemetostat showed an additive effect on latency reversal.

8. Analysis of the HIV-associated gut microbiome

Aya Ishizaka, Michiko Koga, Taketoshi Mizutani[2], Prince Kofi Parbie[3], Diki Prawisuda, Nozomi Yusa[6], Ayako Sedohara, Tadashi Kikuchi[3], Kazuhiko Ikeuchi, Eisuke Adachi[1], Tomohiko Koibuchi, Yoichi Furukawa[6], Arinobu Tojo[7], Seiya Imoto[8], Yutaka Suzuki[2], Takeya Tsutsumi, Hiroshi Kiyono[9], Tetsuro Matano[3,10], Hiroshi Yotsuyanagi

[6] Department of Applied Genomics, IMSUT hospital, IMSUT

[7] Department of Laboratory Medicine, IMSUT hospital, IMSUT

[8] Division of Health Medical Data Science, Health Intelligence Center, IMSUT

9 International Research and Development Center for Mucosal Vaccines, IMSUT

[10] Department of AIDS Vaccine Development, IMSUT Hospital, IMSUT

Loss of gut mucosal barrier function persists during HIV infection and allows translocation of gut-derived bacteria as well as microbial products into circulation. In this study, we aimed to understand compositional changes in gut microbiome and its role in chronic inflammation in Japanese people living with HIV infection (PLWH). We obtained fecal samples from 115 Japanese PLWH and 68 uninfected Japanese controls. Among PLWH, 112 had been treated with ART for more than 16 months and the remaining 3 were ART naïve. The microbiome was characterized by sequencing of the 16S rRNA V3-V4 region. We observed a reduced alpha-diversity of gut microbiome in PLWH with low CD4 count (<250 cells/μl, n=10, P=0.04) but not in those with high CD4 counts (>500 cells/ul, n=105) compared to uninfected controls. Furthermore, inter-group dissimilarity of bacterial composition was observed between PLWH with high CD4 count and uninfected controls. PLWH with high CD4 counts exhibited a significant abundance of bacteria groups in the classes of Negativicutes, Bacilli, and Coriobacteriia compared to uninfected controls. Among PLWH, the abundance of these bacterial taxa correlated positively with inflammatory cytokines such as IL-1β and IFN-γ; But correlated negatively with several anti-inflammatory cytokines including IL-19 and IL-35. Our observation confirmed that decreased bacterial richness and evenness in Japanese PLWH were linked to low CD4 counts. After CD4 recovery, alpha-diversity was restored, but bacterial composition of gut microbiota differed from that of uninfected controls. Notably, bacterial taxa enriched in PLWH were associated with inflammatory cytokine profiles, suggesting the relevance between gut dysbiosis and chronic inflammation in PLWH.

9. Exploration of drugs to restore mitochondrial dysfunction by hepatitis C virus

Takeya Tsutsumi, Kazuhiko Ikeuchi, Diki Prawisuda, Kazuya Okushin[11], Kyoji Moriya[11], Kazuhiko Koike[12], Hiroshi Yotsuyanagi

[11]Department of Infectious Control and Prevention, Graduate School of Medicine, The University of Tokyo

[12]Department of Gastroenterology, Graduate School of Medicine, The University of Tokyo

HCV infection is closely associated with hepatocellular carcinoma (HCC) development, and dysfunction of mitochondria and subsequent reactive oxygen species (ROS) accumulation by HCV, especially the core protein, may contribute to the pathogenesis. So far, we found that the core protein disrupts the function of a mitophagy receptor Bnip3 by suppressing the dimerization of Bnip3, therefore, we are investigating candidate chemicals which rescue the function of mitochondria by using HCV core-transgenic mice, which may lead to the improvement or prevention of the unfavorable pathogenesis. In addition, since the changes in the microbiome are closely associated with pathogenesis in several diseases, we are also investigating the microbiome in the transgenic mice. If significant changes are present, we will attempt to administer probiotics to improve the dysbiosis, to examine if the restoration of the dysbiosis may improve the pathogenesis observed in the transgenic mice. In fact, we started to administrate glycyrrhizin which is known to induce autophagy and are under

investigation of changes in phenotype and microbiome of HCV core-transgenic mice.

10. Clinical epidemiology of malaria in pregnancy

Makoto Saito

Malaria is the leading cause of mortality in the tropics. Pregnant women are particularly vulnerable and malaria in pregnancy causes an adverse impact on the mother and fetus. In the global collaboration with the colleagues in sub-Saharan Africa and Asia, we have conducted a pooled meta-analysis of the clinical data to assess the safety of antimalarials in pregnancy. Currently, we have started another project using the pharmacological data of a clinical trial conducted in Thailand, aiming to optimize the antimalarial drug for pregnant women.

11. Analysis of the gut dysbiosis in patients with HIV infection who developed acute hepatitis A

Aya Ishizaka, Michiko Koga, Taketoshi Mizutani[2], Lay Ahyoung Lim[1] Eisuke Adachi[1], Kazuhiko Ikeuchi, Ryuta Ueda[13], Haruyo Aoyagi[13], Satoshi Tanaka[14], Hiroshi Kiyono[9], Tetsuro Matano[3,10], Hideki Aizaki[13], Sachiyo Yoshio[15], Eiji Mita[14], Masamichi Muramatsu[13], Tatsuya Kanto[15], Takeya Tsutsumi, Hiroshi Yotsuyanagi.

[13] Department of Virology II, National Institute of Infectious Diseases

[14] Department of Gastroenterology and Hepatology, National Hospital Organization Osaka National Hospital

[15] The Research Center for Hepatitis and Immunology, National Center for Global Health and Medicine

Hepatitis A virus (HAV) causes transient acute infection, and little is known of viral shedding via the duodenum and into the intestinal environment, including the gut microbiome, from the period of infection until after the recovery of symptoms. We used blood and stool specimens from patients with HIV who were infected with HAV during the HAV outbreak in Japan in 2018. We observed changes in fecal HAV RNA and quantified the plasma cytokine level and gut microbiome by 16S rRNA analysis from clinical onset to at least 6 months after healing. HAV was detected from clinical onset up to a period of more than 150 days. Immediately after infection, many pro-inflammatory cytokines were elicited, and some cytokines showed different behaviors. The intestinal microbiome changed significantly after infection (dysbiosis), and the dysbiosis continued for a long time after healing. These observations suggest that the immunocompromised state is associated with prolonged viral shedding into the intestinal tract and delayed recovery of the intestinal environment.

12. Analysis of the COVID-19-associated gut microbiome

Taketoshi Mizutani[2], Aya Ishizaka, Michiko Koga, Kazuhiko Ikeuchi, Makoto Saito, Eisuke Adachi[1], Seiya Yamayoshi[5], Kiyoko Iwatsuki-Horimoto[5], Atsuhiro Yasuhara[5], Hiroshi Kiyono[9], Tetsuro Matano[3,10], Yutaka Suzuki[2], Takeya Tsutsumi, Yoshihiro Kawaoka[5], Hiroshi Yotsuyanagi

There is growing evidence that the commensal microbiota of the gastrointestinal and respiratory tracts regulates local and systemic inflammation (gut-lung axis). COVID-19 is primarily a respiratory disease, but the involvement of microbiota changes in the pathogenesis of this disease remains unclear. Currently, we are performing 16S rRNA metagenomic analysis of fecal samples obtained from hospitalized COVID-19 patients. The levels of pro-inflammatory cytokines are also measured and analyzed the relationship between gut dysbiosis during acute COVID-19.

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Advanced Clinical Research Center

Division of Clinical Genome Research
臨床ゲノム腫瘍学分野

Professor	Yoichi Furukawa, M.D., Ph.D.	教　授	博士(医学)	古　川　洋　一
Associate Professor	Tsuneo Ikenoue, M.D., Ph.D.	准教授	博士(医学)	池　上　恒　雄
Senior Assistant Professor	Kiyoshi Yamaguchi, Ph.D.	講　師	博士(薬学)	山　口　貴世志
Assistant Professor	Kiyoko Takane, M.D., Ph.D.	助　教	博士(医学)	高　根　希世子

Research Projects
The aim of our research is the application of findings from basic cancer research to clinics. Currently, we are working on the following five projects, 1) understanding of the role of Wnt/β-catenin signaling pathway in gastrointestinal carcinogenesis, 2) discovery of Wnt antagonists through a screening of large-scale chemical libraries, 3) establishment and investigation of mouse models of human cancer, 4) understanding the genetic features of rare cancers and the mechanisms of their development, and 5) clinical sequencing for the implementation of genomic medicine.

1. Understanding the role of Wnt/β-catenin signaling pathway in gastrointestinal carcinogenesis

Kiyoshi Yamaguchi, Yoichi Furukawa

Aberrant activation of the Wnt/β-catenin signaling pathway has been found in the vast majority of colorectal cancer. This activation leads to the accumulation of β-catenin in the nucleus, where it functions as a transcriptional co-activator of the TCF/LEF family. Therefore, comprehensive understanding of genes directly transactivated by the heterodimeric β-catenin/TCF transcriptional complex will lead to the better understanding of the role of this pathway in human carcinogenesis. Previously, our transcriptome and ChIP-seq analyses using colorectal cancer cells identified five potential genes including *PDE4D*, *PHLDB2*, *MOSPD1*, *FRMD5*, and *OXR1* that are downstream in the Wnt/β-catenin signaling pathway. We extensively analyzed the regulatory mechanism of *MOSPD1* (motile sperm domain containing 1) by the pathway, and found a β-catenin/TCF-response region located in its 3′-flanking region by ChIP-seq data and subsequent reporter assays. An additional chromatin conformation capture (3C) assay implied that the 3′-flanking region interacts with the *MOSPD1* promoter region through a formation of chromatin loop. These data suggested that *MOSPD1* is transcriptionally regulated by the β-catenin/TCF7L2 complex through the enhancer located in the 3′-flanking region. In addition, we confirmed that MOSPD1 expression was frequently elevated in colorectal tumor cells with accumulated β-catenin. Therefore, understanding of the biological role of MOSPD1 will uncover a new role of the pathway involved in carcinogenesis.

2. Discovery of Wnt antagonists through a screening of large chemical library

Kiyoshi Yamaguchi, Yoichi Furukawa, Yoshitaka Ohishi, Satoru Nagatoishi[1], Kouhei Tsumoto[1,2,3]: [1]Project Division of Advanced Biopharmaceutical Science, [2]Medical Proteomics Laboratory, IMSUT, [3]Department of Bioengineering, School of Engineering, The University of Tokyo

A variety of cell-based assays have contributed to the discovery of small molecules that modulate Wnt signaling. Previously, we developed a sensitive and specific cell-based reporter assay for the detection of the Wnt/β-catenin signaling activity. By leveraging

this assay, we established a high-throughput screening system, and performed a screening of small molecule and natural compound libraries. As a result, we have identified several hit compounds for Wnt inhibitors. With the support of the Basis for Supporting Innovative Drug Discovery and Life Science Research (BINDS) program, we have investigated the structure-activity relationship (SAR) of these chemical probes. In addition, we have started gene expression-based approach to elucidate the mode of action of these hit compounds.

3. Establishment and investigation of novel mouse models of human cancer

Tsuneo Ikenoue, Yoichi Furukawa

Genetically engineered mice are useful tools for studying human diseases, including cancer. In this project, we have established a novel mouse model of intrahepatic cholangiocarcinoma (ICC) using liver-specific expression of oncogenic *Kras* and homozygous *Pten* deletion. We have also established another ICC mouse model carrying a cancer-associated mutant allele of *Fbxw7* in combination with an oncogenic *Kras* allele.

In addition, we have investigated roles of cancer-associated hotspot mutations of *IDH1* and *IDH2* in hepatocarcinogenesis using liver-specific knockin mice of these mutations. We also have shown that *IDH1* and *IDH2* mutations induce Glut1 expression and glucose metabolism disorders via activation of PI3K-Akt-mTORC1-Hif1a axis. Intensive analysis of these mice should provide better understanding of mechanisms of carcinogenesis associated with *IDH1/2* mutations and facilitate the development of new therapies to tumors carrying these mutations.

4. Elucidation of genetic characteristics of rare cancers and the mechanisms of their development

Kiyoko Takane, Kiyoshi Yamaguchi, Tsuneo Ikenoue, Yoichi Furukawa, Kotoe Katayama[1], Seiya Imoto[1], Satoru Miyano[2], Hideaki Yano[3], Atsushi Kaneda[4]: [1]Division of Health Medical Intelligence, Human Genome Center, IMSUT, [2]Systems Biology for Intractable Diseases, Medical Research Institute, Tokyo Medical and Dental University, [3]Department of Surgery, National Center for Global Health and Medicine, [4]Department of Molecular Oncology, Graduate School of Medicine, Chiba University

Pseudomyxoma peritonei (PMP) is a rare disease with an incidence of 1 – 2 cases per million and characterized by the presence of mucin-producing tumors in the abdominal cavity. Primary tumors of PMP develop most frequently in the appendix and occasionally in other organs including the ovary, colorectum, gallbladder, stomach, pancreas, fallopian tube, urachus, lung, and breast. To elucidate the molecular mechanisms underlying PMP, we previously analyzed 18 PMPs in appendix by targeted sequencing using the Cancer Hotspot Panel. Consequently, we found that *KRAS* and/or *GNAS* mutations are common genetic features of PMP. In addition, we suggested that mutations in *TP53* and/or genes related to the PI3K-AKT pathway might render malignant properties to PMP.

In addition to genetic changes, we have started a methylome analysis of 7 PMP samples including 5 appendiceal and 2 ovarian PMPs using Infinium 850K BeadChip. Hierarchical clustering analysis using these PMP methylation data and additional methylation data from five non-tumorous colonic mucosa and two normal ovary cases in the Gene Expression Omnibus (GEO) repository, disclosed that PMP was stratified into two epigenotypes, unique-methylation and normal like-methylation epigenotypes. Integrated analysis of these data will facilitate the discovery of biomarkers of PMP, selection of effective anti-cancer drugs, and personalized medical care.

5. Clinical sequencing for the implementation of genomic medicine

Kiyoko Takane, Kiyoshi Yamaguchi, Tsuneo Ikenoue, Yoichi Furukawa, Eigo Shimizu[1], Rika Kasajima[1], Kotoe Katayama[1], Seiya Imoto[1], Tetsuo Shibuya[2], Kazuaki Yokoyama[3], Yasuhito Nanya[3], Koichiro Yuji[4], Rui Yamaguchi[5], Satoru Miyano[6]: [1]Division of Health Medical Intelligence, [2]Division of Medical Data Informatics, Human Genome Center, [3]Department of Hematology/Oncology, [4]Project Division of International Advanced Medical Research, Advanced Clinical Research Center, IMSUT, [5]Division of Cancer Systems Biology, Aichi Cancer Center Research Institute, [6]Systems Biology for Intractable Diseases, Medical Research Institute, Tokyo Medical and Dental University

The application of Next-Generation Sequencing (NGS) technology in clinical medicine has revolutionized molecular diagnostics by enabling multiple gene testing, or analysis of the entire exon or whole genome with a limited amount of DNA. In collaboration with Human Genome Center and Advanced Clinical Research Center, we have been working on the genetic diagnosis of patients with suspected hereditary cancer predisposition, and the implementation of precision medicine for patients with rare or intractable cancer.

We have applied NGS technology for molecular diagnostics of hereditary colon cancer syndromes such as familial adenomatous polyposis (FAP), polymerase proofreading-associated polyposis (PPAP), and Lynch syndrome (LS). LS, also known as hereditary nonpolyposis colorectal cancer syndrome (HN-

PCC), is the most common cause of hereditary colon cancer. Germline variants in the mismatch repair (MMR) genes are responsible for the disease. In the collaborative study with the Japanese Society for Colorectal Cancer (JSCCR), we previously identified substantial number of structural variations (SVs) in the MMR genes. Since detection of SVs using short-read NGS is a challenging work, we took advantage of long-read sequencing technology using Oxford Nanopore MinION device, and further tested the strategy of long-read sequencing coupled with hybridization-based enrichment system for the efficient and accurate detection of breakpoints. We selected four test cases – three with deletion ranging from 1.2 kb to 109.2 kb and one with 8.5 kb duplication in the MMR genes. This approach successfully detected all these SVs with accurate positions of the breakpoints. In addition, we newly identified a deletion across an 84 kb region of *MSH2* in a LS patient without pathogenic single nucleotide variants. These data suggest that long-read sequencing will help the identification of pathogenic SVs in patients with hereditary diseases.

We have been also working on the implementation of genomic data in clinics. An outpatient clinic service in IMSUT hospital offered the consultation of patients with rare or intractable cancer. Patients with colorectal, breast, uterine, pancreatic cancer, and angiosarcoma gave written informed consent for genetic analysis and prediction of treatment using artificial intelligence were enrolled in this study. Genetic alterations in their tumors were determined by NGS, and the data were subsequently analyzed by QIAGEN Clinical Insights (QCI). The results of QCI including predicted driver mutations and suggested actionable drugs were discussed in the Tumor Board which consists of physicians, medical oncologists, genetic counsellors, geneticists, bioinformaticians, and experts of ethics.

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Advanced Clinical Research Center

Division of Innovative Cancer Therapy
先端がん治療分野

Professor	Tomoki Todo, M.D., Ph.D.	教　授	博士(医学)	藤　堂　具　紀
Project Associate Professor	Minoru Tanaka, M.D., Ph.D.	特任准教授	博士(医学)	田　中　　実
Assistant Professor	Hirotaka Ito, M.D., Ph.D.	助　教	博士(医学)	伊　藤　博　崇
Assistant Professor	Yoshinori Sakata, M.D., Ph.D.	助　教	博士(医学)	坂　田　義　詞
Assistant Professor	Yuta Takeshima, M.D., Ph.D.	助　教	博士(医学)	竹　島　雄　太

Our Laboratory is focused on developing oncolytic virus therapies for various malignant tumors. Oncolytic viruses are engineered to kill tumor cells without affecting normal cells. G47Δ, a recombinant, triple-mutated oncolytic herpes simplex virus type 1 (HSV-1), exhibits potent anti-tumor efficacy while maintaining safety. G47Δ was finally approved as the world's first oncolytic virus product for brain tumors in June and is now in clinical use since November 2021.

Development of novel recombinant oncolytic HSV-1

With a steady increase in cancer mortality, there has been a strong need for novel therapeutics for cancers. Oncolytic virus therapy utilizing genetically engineered virus can not only destroy tumor cells by its lytic effect but also shows robust antitumor effect by eliciting systemic tumor-specific antitumor immunity, and is expected as a novel therapeutic for cancer. Various kinds of virus have been modified and utilized as oncolytic viruses, but genetically engineered HSV-1 is particularly useful because of following favorable characteristics: (1) a high specificity for tumor cells while maintaining safety to normal tissues, (2) a high stability of viral genome, (3) a potent oncolytic activity in a wide range of cancer cells, (4) minimally affected by antiviral antibodies on cell-to-cell spread of the virus, (5) a safety net against undesired events by utilizing antiviral drugs, (6) a high capacity for large or multiple transgenes owing to its large genome size of virus (<152kb). We developed G47Δ, an oncolytic HSV-1 with triple gene mutations with high efficacy and safety. While conventional homologous recombination techniques had required time-consuming processes to create a new recombinant oncolytic HSV-1, our original recombinant HSV-1 construction system, T-BAC, enabled quick and accurate generation of a new recombinant HSV-1 with desired transgenes inserted into a specific locus by utilizing two sets of recombinases (Cre/loxP and FLP/FRT).

Translational research of G47Δ was performed totally by this laboratory, including invention, preclinical tests, clinical lot manufacturing and clinical trials since 2003. G47Δ was finally approved as the world's first oncolytic virus product for malignant brain tumors in 2021. Besides malignant brain tumors, we have meticulously accumulated pre-clinical data with the intention to expand the application of G47Δ for other cancers, including renal cancer, prostate cancer, bladder cancer, malignant mesothelioma, tongue cancer, esophageal cancer, gastric cancer, colon cancer, lung cancer, breast cancer, nasopharyngeal cancer, cholangiocarcinoma, hepatic cancer, pancreatic cancer, malignant melanoma, and malignant lymphoma.

Preclinical research has revealed that G47Δ is universally effective for all types of solid tumors, and is expected as an innovative treatment for cancer in the near future. The clinical trials of G47Δ for malignant mesothelioma, olfactory neuroblastoma and prostate

cancer, and that of human IL-12-expressing G47Δ (T-hIL12) for melanoma have been proceeding and will soon advance to the next phases of clinical trials.

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Advanced Clinical Research Center

Division of Advanced Medicine Promotion
先端医療開発推進分野

Professor	Fumitaka Nagamura, M.D., D.M.Sc	教　授　博士(医学)	長　村　文　孝
Associate Professor	Masanori Nojima, M.D., Ph.D., M.P.H.	准教授　博士(医学)	野　島　正　寛

Our mission is to assist the development of translational research. For this purpose, it is critical to discover new "seeds" and to eradicate blockades until the clinical utilization. We also assist the conduct of clinical trials at IMSUT Hospital. At IMSUT Hospital, we work together with staffs of Center for Translational Research. Concurrently, to concur blockades on translational research, we have been engaging in research on regulatory science and biostatistics.

1. Assistance of Clinical Trials/TRs at Research Hospital

Minako Kouno, Riyo Owada, Masanori Nojima, Fumitaka Nagamura

At IMSUT Hospital, we work together with staffs of Center for Translational Research. The assistance of Translational (Clinical) Research Coordinators is indispensable for the conduct of clinical trials, especially for TR. The activities of Coordinators are results of the collaboration between Division of Advanced Medicine Promotion and Center for Translational Research. In 2021, we supported 5 sponsor-investigator clinical trials and 3 non-IND clinical studies.

2. Management of "Translational Research Network Program" of Japan Agency for Medical Research and Development.

Miwako Okada, Fumitaka Nagamura

Ministry of Education, Culture, Sports, Science and Technology launched "Translational Research Network Program" to promote translational research based on the results of basic science at academia. This grogram was transferred to Japan Agency for Medical Research and Development in 2015 and has been expected to support TRs from basic science to seek obtaining intellectual property to early stage of clinical trial. In 2021, we supported 21 basic researches (13: other than IMSUT), 18 preclinical studies (10: other than IMSUT), and 11 clinical studies (4: other than IMSUT). The number of studies we assist has been increasing year by year. Organization reinforcement is the urgent problem.

3. Approach for epigenome and multi-omics research by methodology of bioinformatics and biostatistics

Masanori Nojima

Epigenome and multi-omics research using clinical samples in collaborative study or public database of comprehensive omics-analysis. We are now focusing on the multi-omics approach integrating DNA methylation, mRNA expression, and miRNA, and building statistical models to assess functional linkage.

4. Statistical consulting

Masanori Nojima

Consulting for study design and statistical analysis in any type of clinical research including clinical research, basic medical/biological research. We have collaborated with other members in IMSUT and other institutions through the consulting.

5. Statistics and Quality control in Clinical Trials

Masanori Nojima, Motoki Amai, Mitsumi Tokunaga, Fumitaka Nagamura

We have planned and performed data management, monitoring, and statistical works in clinical trials.
[Data management]: Planning, EDC and CRF preparation, registration, allocation, database management, data cleaning, coding
[Monitoring]: Monitoring for drug management
[Statistics]: Planning and perform for statistical analyses, Sample size calculation.

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Advanced Clinical Research Center

Division of Advanced Genome Medicine
先端ゲノム医学分野

Associate Professor Yoshihiro Hirata, M.D., Ph.D. | 准教授 博士(医学) 平 田 喜 裕

The goals of our researches are to identify the mechanisms and to establish novel therapies especially for cancers and inflammatory diseases of the digestive system. One of the research fields is the inflammatory diseases, in which we investigated the molecular pathogenesis of gastritis, cholangitis and inflammatory bowel disease. Another research field is the malignancies. We specifically focus on the topics such as, differentiation of stem cells, proliferation and death of epithelium, interactions with immune cells or microbes, inter-organ interactions, and maintenance of tissue homeostasis. Using genetically engineered mice, we try to unveil the pathogenesis of various digestive diseases.

1. Role of IL-33 in the gastrointestinal homeostasis

Yoshihiro Hirata, Kazuya Koyanagi, Yuka Kurihara, Aya Yamashita[1], Nobumi Suzuki[1], Keisuke Tateishi[1]. [1]Department of Gastroenterology, The University of Tokyo

To investigate the role of IL-33 in intestinal tract, we have established conditional IL-33 expression mouse (LSL-IL-33 mouse) and crossed with GI-tract specific cre expression mice line. Stomach specific IL-33 expression (TFF1-cre-LSL-IL33, Mist1creERT-LSL-IL33) induced gastritis characterized by CD11b+ myeloid cell infiltration not only in the lamina propria, but also in the muscular layer and serosa. Gastric epithelium showed loss of parietal cells and chief cells indicating gastric atrophy with metaplasia. In clear contrast, intestine specific IL-33 expression (villin-LSL-IL33) did not exhibit inflammatory phenotype. This specific effect of IL33 on gastric tissue is also confirmed by CRISPR-Cas9 mediated IL-33 knock-in mouse line (TFF1pro-IL33). The mice established in this study will be useful as a research tool of specific gastritis.

2. Role of Sox9 in the gastric carcinogenesis

Kazuya Koyanagi, Aya Yamashita[1], Nobumi Suzuki[1], Keisuke Tateishi[1], Yoshihiro Hirata. [1]Department of Gastroenterology, The University of Tokyo

Sox9 is a multifunctional transcriptional factor which participates in development, stemness, as well as carcinogenesis of various tissues. We previously found that gastric epithelial cells of H. pylori-infected gastritis express high level of Sox9. To elucidate the role of Sox9 in gastric diseases, we now established stomach specific Sox9 knockout mice and started experiments using this mouse model.

3. Pathogenesis of squamo-columnar junction cancer of the stomach

Yoshihiro Hirata

Squamo-columnar junction (SCJ) is one of the transitional zones in body where two different cell types merge. We have established a mouse model in which invasive tumors developed specifically at gastric SCJ by the expression of oncogenic Ras and deletion of TGFbR2. This tumor is characterized by adeno-squamous character by histology and heterogenous expression of SOX2 or SOX9, and KRT19 or KRT5 by IHC, suggesting the presence of SCJ specific bi-directional tumor initiating cells.

4. Role of acetylcholine signaling in the inflammatory bowel diseases

Aya Yamashita[1], Yoshihiro Hirata, Sozaburo Ihara[2], Yoku Hayakawa[1], Hayato Nakagawa[1], Kazuhiko Koike[1], Lars Eckmann[2]. [2]Department of Gastroenterology, Faculty of Medicine, University of California, San Diego

We examined the role of acetylcholine signaling in colitis using murine colitis model. Administration of nicotine, a ligand of nicotinic acetylcholine receptor, to IL-10 knockout mice reduced inflammatory cell infiltration and retained goblet cells. In vitro, nicotine treatment attenuated cystic dilatation of WT DC co-cultured intestinal organoids, but not of alpha7nicotinic acetylcholine receptor KO DC co-cultured organoids. IL-10 KO mice with DC-specific alpha7nicotinic acetylcholine receptor deletion exhibited more severe colitis than IL-10 KO mice indicating the importance of acetylcholine signaling on DCs.

5. Molecular mechanism of the development and the progression of sclerosing cholangitis

Hisayoshi Natomi, Hayato Nakagawa, Yoshihiro Hirata

Primary sclerosing cholangitis is a rare form of biliary inflammation which can progress to cirrhosis and cancer. We are currently investigating the effects of life style factors, such as smoking and obesity, as well as the role of intestinal microflora, on cholangitis using originally developed mouse biliary disease models. We found infiltrated T cells have Th17 signatures, and damaged epithelium express stem cell markers. Antibiotics treatment ameliorated immune cell infiltration and fibrosis of bile duct.

6. The role of fusion HBx from HBV integrant in the hepatocarcinogenesis

Ryosuke Muroyama, Naoya Kato, Yoshihiro Hirata

We identified fusion HBx translated from HBV integrant in human hepatocellular carcinoma cell line. In HBx KD cells, cell proliferation, invasion ability as well as tumor formation in nude mice, were significantly reduced. The fusion HBx had anchorage-independent growth ability in soft agar although the fusion HBx completely abrogated its transactivation ability. We also found that the fusion HBx dysregulated ER stress response via the modification of ATF3, ATF4, and ATF6 transcription.

Publications

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Advanced Clinical Research Center

Division of Bioethics
生命倫理研究分野

Associate Professor Ayako Kamisato, Ph.D.

准教授　博士（法学）　神　里　彩　子

Medical research has high expectations from society, however, it imposes burdens and risks on subjects. And also, research using advanced medical technology may raise new ethical, legal and social issues (ELSI). For these reasons, certain rules are required for medical research. In our laboratory, we consider what kind of rules are necessary for protecting subjects and responding to the new ELSI, based on the opinions of the general public.

1. The REC Education program for Research Ethics Committees (RECs)

Ayako Kamisato, Kazuyo Arisawa, Hong Hyunsoo

Japanese ethical guidelines "Ethical Guidelines for Medical and Biological Research Involving Human Subjects" and "Regulation for Enforcement of Clinical Research Act" now mandate that institutions which established RECs should offer education and training programs to REC members at least once a year. However, the guidelines and regulation do not make any provisions regarding the contents of programs and the way of implementation. As implementation of education and training programs require manpower and economic resources, most institutions are unable to provide high-quality education and training. To address this situation, we have constructed the REC Education program with support from the Japan Agency for Medical Research and Development (AMED) since FY 2016.

Our programs have the following features:1)programs are animated, 2)in order to offer the REC members how to review from their place, we created four characters: two experts in natural science and law, a lay member, and a secretariat, 3)each program has an agenda of discussion, 4)an external expert committee evaluates each program prior to release, 5)each program is about 20 minutes long, 6)the programs are offered at no charge on the website,7)REC which successfully complete the program could receive a certificate of completion.

We have produced following video programs and released on our website:

- Module 1. Revision of the Privacy Act
- Module 2. Procedure of Informed Consent for using human samples and information
- Module 3. Why REC is necessary? What is the role of each REC member?
- Module 4. Checklist for Effective Reviewing
- Module 5. Invasive Research and Interventional Study
- Module 6. Basic knowledge of clinical trials 1
- Module 7. Basic knowledge of clinical trials 2
- Module 8. Basic knowledge of clinical trials 3
- Module 9. Outline of "Clinical Research Act"
- Module 10. The points for reviewing on Informed Consent 1
- Module 11. The points for reviewing on Informed Consent 2
- Module 12. The points for reviewing on Informed Consent 3
- Module 13. The points for reviewing on Informed Consent 4
- Module 14. Handling of personal information
- Module 15. Outline of new "Ethical Guidelines for Medical and Biological Research In-

volving Human Subjects" and the points for reviewing

As new "Ethical Guidelines for Medical and Biological Research Involving Human Subjects" have been enforced in 2021, we also checked all videos and revised them to comply with the new guidelines.

Currently, we have 2058 members and 718 institutions registered with our program as of 15 January, 2022. We constantly assess our programs through questionnaires to improve each program. We have consistently received high scores from our users.

2. The REC Education program for Researchers

Ayako Kamisato, Kazuyo Arisawa, Hong Hyunsoo

"Ethical Guidelines for Medical and Biological Research Involving Human Subjects" and "Regulation for Enforcement of Clinical Research Act "require researchers to receive ethical education and training programs at least once a year. However, there are problems of manpower and economic resources in education and training programs for researchers as well as for REC members. To address these problems, we have produced the REC Education program for researchers with support from the Japan Agency for Medical Research and Development (AMED) since FY 2019. We have already produced and released following video programs.

- Module 1. Clinical Research Act-What kind of research is subject to Clinical Research Act?
- Module 2. Clinical Research Act-Procedures for conducting specific clinical research.
- Module 3. Outline of new "Ethical Guidelines for Medical and Biological Research Involving Human Subjects" (Japanese version)
- Module 4. Outline of new "Ethical Guidelines for Medical and Biological Research Involving Human Subjects" (English version)

Currently, we have 2677 members registered with this program as of 15 January, 2022.

3. Large scale survey on public's awareness of providing samples and medical information for medical research

Ayako Kamisato, Kazuyo Arisawa

From the perspective of promoting data utilization, we conducted an Internet survey of the general public in Japan in order to investigate awareness of sample provision and profit sharing on the premise of secondary use of data for medical research. We found that about 50% of respondents were positive to provide samples for medical research. The results also showed about 50% of the respondents believed that the samples would belong to researchers and research institutes once those were provided, however, it turned out that nearly 60% of the respondents thought that financial benefits should be returned to the providers. We are currently preparing for the dissertation submission.

4. Survey of public attitude on triage in Covid-19 pandemic

Ayako Kamisato

Kamisato conducted an internet survey to see the public's understanding and attitude on using a ventilator or ECMO in Covid-19. The results showed that many people were unaware that they could not communicate when using a ventilator due to sedation. This suggested that many patients were used ventilators without telling doctors or families what they needed to do, such as expressing their intentions about treatment or giving messages to their families. Results of this survey were covered by many media and contributed to the promotion of public understanding of ventilators and ECMO.

5. Policy making of research using genome editing technology for human embryos

Ayako Kamisato

The Expert Panel on Bioethics of Council for Science, Technology and Innovation at Cabinet Office set research using genome editing technology for human embryos as an agenda. Kamisato participated as a member of the Expert Panel and contributed for policy making. She is also a member of the Council on research using genomic editing technology for human fertilized embryos set by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) and Health, Labour and Welfare Ministry (MHLW) and contributed for revising "Ethical guidelines for research using genetic information modification technology for fertilized human embryos".

6. Production of tools for researchers who use genetic information modification technology for fertilized human embryos

Ayako Kamisato

Kamisato produced educational video for researchers on "Ethical guidelines for research using genetic information modification technology for fertilized human embryos" and common IC Form with support from the Japan Agency for Medical Research and Development (AMED). Video is now released on the Ministry of Education, Culture, Sports, Science and Technology (MEXT) website.

Publications

- 佐藤桃子, 神里彩子, 武藤香織「出世前遺伝学的検査における用語「マススクリーニング」使用に関する言説分析」日本遺伝カウンセリング学会誌 42(3) 307-317 （2021年11月）

Advanced Clinical Research Center

Division of Frontier Surgery
フロンティア外科学分野

Professor	Dai Shida, M.D., Ph.D.	教　授　博士(医学)	志　田　　大
Associate Professor	Susumu Aiko, M.D., Ph.D.	准教授　博士(医学)	愛　甲　　丞
Assistant Professor	Yuka Ahiko, M.D.	助　教	阿　彦　友　佳

The mission of our division is to create solid evidence of surgical treatment for colorectal cancer as well as gastric cancer by continuously publishing the papers of clinical research and basic research. If our research can help rewrite various clinical guidelines around the world, we as surgeons can not only cure the patients in front of us but also contribute to the development of surgical treatment for gastrointestinal cancer.

1. Introduction

This division was newly established in September 2020 by professor Shida and Dr. Ahiko. On November 16th, professor Dr. Aiko also joined this new division. We named our new division as 'Frontier Surgery', because we want to greedily open up undeveloped areas of surgery and contribute to the development of surgery.

2. Treatment for diseases of colon, rectum, anus and stomach in IMSUT hospital

All of us are also members of Department of Surgery, IMSUT hospital. We treat diseases of colon, rectum, anus, and stomach, especially colorectal cancer and gastric cancer.

See NO16-10 (Department of Surgery, IMSUT Hospital).

3. Making Evidence for gastrointestinal malignancy

While performing surgery as gastrointestinal surgeons, we are planning to conduct translational research as academic surgeons in the near future.

4. Publications (as a member of IMSUT)

Horie T, Shida D, Ahiko Y, Takamizawa Y, Inoue M, Tanabe T, Nakamura Y, Imaizumi J, Tsukamoto S, Kanemitsu Y.

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Advanced Clinical Research Center

Division of Hematopoietic Disease Control
造血病態制御学分野

Professor	Yasuhito Nannya, M.D., Ph.D.	教　授　博士(医学)	南　谷　泰　仁
Associate Professor	Takaaki Konuma, M.D., Ph.D.	准教授　博士(医学)	小　沼　貴　晶

The main purpose of our research is to elucidate the pathogenesis of hematopoietic diseases and to study the development of therapies for these diseases. For those studies for which we have already determined the therapeutic targets, we will steadily advance the development and proceed to the next stage with clinical application. Specifically, this includes the development of therapies for myeloid tumors targeting Immunoglobulin Superfamily Member 8 and NK cell therapy using CD155/CD112, which are immune checkpoint molecules.
Additionally, we are leading a project for whole genome sequencing of more than 1,400 leukemia samples collected from major institutions that treat hematopoietic diseases in Japan, and the analysis of the genome data of these samples will start this year. In this study, we will comprehensively search for abnormalities occurring in non-gene-coding regions of the genome and beyond, with the aim of elucidating previously undiscovered pathological mechanisms. In addition, we aim to identify the genetic predisposition to the development of leukemia.
Furthermore, in order to promote the elucidation of novel pathogenesis, we are developing a platform to advance the subclassification of diseases based on susceptibility using a drug susceptibility screening system and to elucidate the mechanisms of susceptibility pathogenesis by multi-omics analysis.

1. Immunoglobulin Superfamily Member 8 is indispensable for myeloid leukemia via Wnt/β-catenin signaling pathway

Jimbo K[1], Konuma T[1,2], Ito T[3], Nakajima-Takagi Y[4], Iwama A[4], Tojo A[2,5], Yasuhito Nannya[1,2],
1 Division of Hematopoietic Disease Control
2 Department of Hematology/Oncology, IMSUT Hospital
3 Laboratory of Cell Fate Dynamics and Therapeutics, Institute for Frontier Life and Medical Sciences, Kyoto University
4 Division of Stem Cell and Molecular Medicine,
5 Division of Molecular Therapy

Immunoglobulin superfamily member 8 (IGSF8, EWI-2, or CD316), a cell surface protein containing 4 IG domains, interacts with several tetraspanins including CD9 and CD81, and modulates cell migration and motility. We examined the role of Igsf8 in normal hematopoiesis and leukemogenesis using conditional knockout mice (Igsf8 f/f; Vav-Cre or Igsf8 f/f; Rosa26-CreERT). Igsf8 was ubiquitously expressed in normal blood cells and leukemia cells. Igsf8-/- did not significantly affect adult hematopoiesis in peripheral blood and bone marrow. Igsf8-/- LT-HSC (CD34-Flk2- KSL cells) reduced colony forming ability in vitro, and showed comparable donor chimerism by 3 months, but led to reduced donor chimerism at 4 months and those after second transplantation in vivo, suggesting that Igsf8 does not affect adult hematopoiesis, but that may affect repopulating ability of HSCs. In MLL-AF9 and NRASG12V-driven AML, or BCR-ABL and NUP98-HOXA9-driven CML-BC

mice models, Igsf8-/- led to a dramatic decrease in the number of leukemic colonies formed in vitro. Igsf8-/- leukemia mice showed significantly longer survival and depleted leukemia cells in bone marrow and spleen in vivo. Igsf8-/- leukemia cell triggered an increment of apoptotic cell death, which contribute to significantly lower proportion of LSCs in spleen of Igsf8-/- leukemic mice. Given that Igsf8-/- did not affect homing ability of leukemia cells, these results indicate that Igsf8 is required for propagation of myeloid leukemia and maintenance of LSC. Gene set enrichment analysis exhibited increase apoptosis related genes and decrease Wnt/β-catenin related genes in Igsf8-/- leukemic cells, but not in LT-HSCs. Knockdown of IGSF8 by small hairpin RNA in myeloid leukemia cell lines (THP-1, MV4-11, SKM-1, and K562) and primary patient-derived AML cells exhibited reduced numbers of colony forming cells and improves the survival of recipients in xenograft models of myeloid leukemia. At a molecular level, we found that activation of β-catenin in LSCs depends on Igsf8, which promotes the association of FZD4 with its co-receptor LRP6 in the presence of Igsf8. Collectively, these data indicate strong genetic evidence identifying Igsf8 as a key regulator of myeloid leukemia and the possibility that targeting IGSF8 may serve as a new therapeutic approach against myeloid leukemia.

2. Elucidation of immune checkpoints CD155/CD112 as possible targets of FLT3 inhibitors in acute myeloid leukemia and generation of genetic engineered NK cells with enhanced anti-tumor cytotoxicity.

Kaito Y[1], Hirano M[1,2], Futami M[1,2], Tojo A[2,3],Yasuhito Nannya[1,2], ImaiY[1,2].
[1]Division of Hematopoietic Disease Control
[2]Department of Hematology/Oncology, IMSUT Hospital
[3]Division of Molecular Therapy

Acute myeloid leukemia (AML) relapse is considered to occur due to escape of tumor cells from anti-tumor immunity and contribution of immune checkpoints CD155/CD112 to AML progression is assumed. However, both the activation receptor DNAM-1 and inhibitory receptor TIGIT present on natural killer (NK) and T cells bind to CD155/CD112. It is unclear how changes in the expression of CD155/CD112 affect tumor immunity. The Raf-MEK-ERK pathway, related to regulation of CD155/CD112 expression, is one of the targets of FLT3 inhibitors. We investigated the effect of FLT3 inhibitors on the expression of CD155/CD112 and its effects on NK and T cell cytotoxicity. CD155/CD112 expression in AML cell lines with or without treatment of the FLT3 inhibitor quizartinib was analyzed. The direct cytotoxicity and antibody-dependent cellular cytotoxicity (ADCC) of NK cells under FLT3 inhibition were determined by luciferase reporter assay. The cytotoxicity of γδ T cells was also analyzed. CD155/CD112 expression was specifically downregulated by the FLT3 inhibitor in FLT3 mutated cell lines. The direct cytotoxicity and ADCC of NK cells were enhanced. However, the cytotoxicity of γδ T cells with decreased TIGIT expression as compared to that of NK cells was not enhanced. Analysis of clinical trials from the database revealed that high CD155/CD112 expression is associated with poor overall survival. The enhanced cytotoxicity of NK cells against the cells that were treated with FLT3 inhibitors suggests that CD155/CD112 are possible target of FLT3 inhibitors in AML. In addition, we tried to generate genetic engineered NK cells with enhanced anti-tumor cytotoxicity. First, we introduced human IL-15 using lentivirus vector into NK-92 cells, NK cell lines derived from human NK cell lymphoma. Although NK-92 cells are cytokine dependent cell lines, the introduction of IL-15 made NK-92 cells proliferative independent of cytokines. This genetic engineered NK cells were supposed to be useful for our future analyses especially in in vivo analyses using patient-derived xenografts (PDX) mouse model. Furthermore, we performed genome editing in NK-92 cells using CRISPR-Cas9 systems. The cytotoxicity of DNAM-1+/TIGIT- NK-92 cells against AML cells with or without FLT3 mutations was enhanced as compared with that of DNAM-1-/TIGIT+ NK-92 cells. These results indicate the usefulness of genome editing of immune check point-related genes in NK cells to enhance their anti-tumor cytotoxicity. We are in the process of genome editing of immune check point-related genes in *induced pluripotent stem* (iPS) cells and differentiation-induction of genome edited iPS cells into NK cells.

3. Reconstitution of circulating mucosal-associated invariant T cells after allogeneic stem cell transplantation; its association with the riboflavin synthetic pathway of gut microbiota in cord blood transplant recipients.

Konuma T1, 2, Kohara C1, Watanabe E3, Takahashi S4, Ozawa G4, Suzuki K5, Mizukami M5, Nagai E5, Jimbo K1, Kaito Y1, Isobe M1,2, Kato S1,2, Takahashi S1,2,Chiba A6, Miyake S6, Tojo A1,7, Yasuhito Nannya1,2
[1]Division of Hematopoietic Disease Control
[2]Department of Hematology/Oncology, IMSUT Hospital
[3]IMSUT Clinical Flow Cytomery Laboratory
[4]TechnoSuruga Laboratory
[5]Department of Laboratory Medicine, IMSUT Hospital
[6]Department of Immunology, Juntendo University School of Medicine
[7]Division of Molecular Therapy

Mucosal-associated invariant T (MAIT) cells are a

type of innate lymphocyte and recognize riboflavin (vitamin B2) synthesis products presented by MHC-related protein 1. We investigated long-term reconstitution of MAIT cells and its association with chronic graft-versus-host disease (cGVHD) in a cross-sectional cohort of 173 adult patients after allogeneic hematopoietic cell transplantation. According to donor source, the number of MAIT cells significantly correlated with time after cord blood transplantation (CBT) but not with time after bone marrow transplantation or peripheral blood stem cell transplantation.

The number of MAIT cells was significantly lower in patients with cGVHD compared with patients without cGVHD. We also examined the association between MAIT cell reconstitution and gut microbiota as evaluated by 16S ribosomal sequencing of stool samples 1 mo post-CBT in 27 adult patients undergoing CBT. The diversity of gut microbiota was positively correlated with better MAIT cell reconstitution after CBT. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States analysis indicated that amounts of *ribB* and *ribA* genes were significantly higher in the microbiomes of patients with subsequent MAIT cell reconstitution after CBT.

In conclusion, long-term MAIT cell reconstitution is dependent on the type of donor source. Our data also unveiled an important role for the interaction of circulating MAIT cells with gut microbiota in humans.

4. Post-azacitidine clone size predicts long-term clinical outcome of patients with myelodysplastic syndromes and related myeloid neoplasms.

Yasuhito Nannya[1,2,3], Magnus Tobiasson[4], Shinya Sato[5], Elsa Bernard[6], Satoru Miyano1[7], Elli Papaemanuil[6], Yasushi Miyazaki[5],Eva Hellström-Lindberg[4] and Seishi Ogawa[3,4]

1 Division of Hematopoietic Disease Control

2 Department of Hematology/Oncology, IMSUT Hospital

3 Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto University

4 Division of Hematology, Department of Medicine, Karolinska University Hospital, Stockholm, Sweden.

5 Department of Hematology, Atomic Bomb Disease and Hibakusha Medicine Unit, Atomic Bomb Disease Institute, Nagasaki University, Nagasaki, Japan

6 Memorial Sloan Kettering Cancer Center, New York, NY, USA

7 Department of Integrated Data Science, M&D Data Science Center, Tokyo Medical and Dental University,

Azacitidine is a mainstay of therapy for MDS and other myelodysplasia. The purpose of our study is to elucidate the effect of gene mutations on hematological response and overall survival (OS), particularly focusing on their post-treatment clone size. We enrolled 341 patients with MDS or related myeloid neoplasms who received azacitidine treatment. They were analyzed for gene mutations in pre- (n = 341) and post- (n = 237) treatment bone marrow samples using targeted-capture sequencing to assess the impact of gene mutations and their post-treatment clone size on treatment outcomes. The results were validated in an independent cohort (n = 110). Even though significantly associated with a higher complete remission rate (odds ratio, 3.38; 95% CI, 1.74 to 6.60; P < .001), TP53 mutations in pre-treatment samples, when present in multi-hit state, predicted a shorter OS (HR, 1.86; 95% CI, 1.22 to 2.83; P = .004). By contrast, DDX41 mutations were associated with a significantly longer OS (HR, 0.30; 95% CI, 0.16 to 0.57; P < .001), although not predicting initial response. These effects were largely reproduced in the validation cohort (n = 110). As expected, the post-treatment clone size significantly correlated with hematological response (P = .0001, trend test) and notably, predicted OS (HR, 1.76; 95% CI, 1.05 to 2.97; P = .032), independently of the IPSS-R risk score, hematological response, and mutation profile. The prognostic model solely based on IPSS-R is improved in a stepwise manner by including initial response, mutation state, and post-treatment clone size in this order, with an increasing c-index from 0.642 to 0.722. Evaluation of post-treatment clone size as well as pre-treatment TP53 and DDX41 mutations has a role in better prognostication for azacitidine-treated myelodysplasia patients.

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Center for Stem Cell Biology and Regenerative Medicine

Division of Regenerative Medicine
再生医学分野

Professor	Hideki Taniguchi, M.D., Ph.D.	教　授	博士(医学)	谷　口　英　樹
Associate Professor	Naoki Tanimizu, Ph.D.	准教授	博士(農学)	谷　水　直　樹
Project Associate Professor	Tomoyuki Yamaguchi, Ph.D.	特任准教授	博士(医学)	山　口　智　之
Assistant Professor	Yun-Zhon g Nie, Ph.D.	助　教	博士(医学)	聶　　運　中
Project Assistant Professor	Yasuharu Ueno, Ph.D.	特任助教	博士(医学)	上　野　康　晴

Currently, organ transplantation is the only effective treatment for patients with end-stage organ failure; Unfortunately, the scarcity of transplantable organs hinders the application of this treatment for most patients. Recently, regenerative medicine prospect on the generation of transplantable organs has attracted much attention. Regenerative medicine is a frontier scientific field that integrating pioneer knowledge on developmental biology and stem cell biology as the foundation for clinical application. Our laboratory aims to develop the latest breakthrough in stem cell therapies as a substitute to the needs of organ transplantation. We have established novel organoid culture technologies to reconstruct human organs outside the body from stem cells, including human induced pluripotent stem cells (hiPSCs). Our latest work on the transplantation of human liver primordia (liver buds [LBs]) generated from hiPSCs aims to treat liver diseases, such as metabolic disorders and liver fibrosis. Recently, we expand the application of our technologies to reconstruct artificial refractory cancer tissue (cancer organoid) with actual tumor microenvironment for developing a new drug-screening platform to discover candidate compounds that could prevent cancer relapse and metastasis.

1. Development of treatment for metabolic liver disease by transplantation of human iPSCLB

Yasuharu Ueno[1], Yunzhong Nie[1], Yoshiki Kuse[1], Takashi Okumura[1], Toshiharu Kasai[1], Eriko Kanai[1], Syusaku Tsuzuki[1], Shinya Matsumoto[1], Yumi Horie[1], Erika Jinbo[1], Satoshi Okamoto[2], Tomomi Tadokoro[2], Takuji Maeda[2], Soichiro Murata[2] and Hideki Taniguchi[1,2]

[1] Division of Regenerative Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo

[2] Department of Regenerative Medicine, Graduate School of Medical Science, Yokohama City University

Hepatocytes play crucial roles in maintaining homeostasis in living organisms by carrying out various metabolic functions including ammonia detoxification. Urea cycle disorders, inherited metabolic deficiencies with hyperammonemia, are caused by a single gene defect of the urea cycle enzymes or transporters. The lack of ornithine transcarbamylase (OTC), a rate-limiting enzyme in the urea cycle, is commonly referred as OTC deficiency (OTCD) and is the most common urea cycle disorder in human. Since iPSC-LBs possess OTC activity, the transplantation of iPSC-LB potentially supports the defective urea cycle in OTC patients and is expected as a novel effective therapy for the treatment of OTCD. To establish a new therapy with human iPSC-LB transplantation for OTCD patients, currently we are verifying the efficacy and safety of iPSC-LB transplantation to meet the

clinical research standard. The renal capsule transplantation of iPSC-LBs demonstrated hepatic functions in immunodeficient OTCD mice through the improvement of hyperammonemia, indicating the transplantation efficacy of iPSC-LBs for treating OTCD.

As the next step, we are now preparing for clinical trials on OTCD patients and considering to expand the target diseases for transplantation treatment using hiPSC-LBs.

To this end, by using Statistical Design of Experiments (DOE) method, we have established a very stable differentiation induction algorithm for the induction of hepatic endoderm cells from human iPSCs. Lastly, in order to improve therapeutic effects of iPSC-LB transplantation, the adjustment on the number of human iPSC-LBs for each transplantation might be required. Therefore, we have developed a method for transplanting hiPSC-LBs on the liver surface, and plan to establish a protocol to reduce the immune response after allogeneic transplantation (*Int J Mol Sci*, 22(21):11589, 2021, *Cells*, 10(2):476.).

2. Effectively massive production of liver organoid with human iPSC derived proliferative progenitors

Yun-Zhong Nie[1], Yang Li[1], Xia Yang[1], Richie Plummer[1], Yoshihito Hayashi[1], Xiao-Shan Deng[1], Eriko Kanai[1], Hideki Taniguchi[1,2]

[1] Division of Regenerative Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo

[2] Department of Regenerative Medicine, Graduate School of Medical Science, Yokohama City University

The development of new transplantable human livers from hiPSCs is urgently needed due to the profound shortage of transplantable donors to treat end-stage liver diseases. Our laboratory has developed three-dimensional (3D) vascularized liver organoids (LOs) from hiPSCs by mimicking the early liver organogenesis. We are currently investigating the potential application of the LOs for treating liver diseases, including inherited metabolic liver disease and liver cirrhosis. To further accelerate the adoption for clinical translation, we aim to establish an efficient, simple, and low-cost culture system to realize the massive production of LOs without iPSC contamination. With a comprehensive understanding of fetal liver development, we successfully generated proliferative hepatoblasts and hepatic stellate cells from human iPSCs. These human iPSC-derived hepatoblasts demonstrated repopulation potential in mouse liver injury models, whereas human iPSC-derived hepatic stellate cells functioned as non-parenchymal cells that support the LOs generation. Moreover, we establish a Matrigel-free and 3D-microwell plate-free method for LOs generation, making it possible to produce a large amount of LOs more efficiently for translational studies. Now, we plan to combine the aforementioned techniques to establish a safe and effective therapeutic strategy for liver diseases.

3. hiPSC-liver bud *in vitro* growth enhanced by perfusion culture

Yoshiki Kuse[1], Naoki Tanimizu[1], Yasuharu Ueno[1], Megumi Matsuo[1], Nie Yunzhong[1], Shinya Matsumoto[1], Takashi Okumura[1], Erica Carolina[1], Soichiro Yamabe[1], Eriko Kanai[1], Syusaku Tsuzuki[1], Toshiharu Kasai[1], Tomomi Tadokoro[2], Satoshi Okamoto[2], and Hideki Taniguchi[1,2]

[1] Division of Regenerative Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo

[2] Department of Regenerative Medicine, Graduate School of Medical Science, Yokohama City University

To overcome the critical shortage of donor organs, the generation of hiPSC-liver with structures and functions similar to the liver is anticipated. Blood perfusion is the critical event for organ growth by supplying nutrients and oxygen. However, tissue perfusion is lacked in the present organoid culture system. We are developing a perfusion culture system through two distinct approaches; First, hiPSC-liver buds (LBs) integrated with an artificial blood vessel, and second, the establishment of decellularized liver tissue containing hiPSC-LBs.

As the first approach, we have generated the hiPSC-derived macrovessel consisting of collagen gels scaffold, hiPSC-derived vascular smooth muscle cells (SMC), and the delineating vascular endothelial cells (EC). We clarified that hiPSC-derived macrovessel is histologically similar to the vascular structure of *in vivo* blood vessels. However, when macrovessels were co-cultured with hiPSC-LBs, ECs within the macrovessels did not show any sign of angiogenesis. Therefore, we established a novel induction method to differentiate hiPSC into a specific lineage of ECs which existed around the fetal liver during liver development. hiPSC-derived macrovessel containing that specific lineage of ECs demonstrated a higher angiogenic potentials. Followingly, we are optimizing a method to initiate the connection between the human iPSC-derived macrovessels and the microvessels within human iPSC-LBs for the establishment of an organoid perfusion system.

As the second approach, we utilized decellularized liver tissue in which *in vivo* vascular structures remain intact. Decellularization technique has been well established as a scaffold for organ reconstitution since the final output material potentially retain the

architecture of the original tissue including the extracellular matrix. A recent report shows that the recellularized liver using hepatocytes exerts liver specific functions after transplantation. However, the vascular structures in that study are not well reconstructed, and hepatocyte functions are very limited. We are now challenging to generate more functional recellularized liver by the infusion of human iPSC-derived LBs into decellularized liver.

4. Generation of 3D cancer tissue using patient-derived pancreatic cancer cells

Tomoyuki Yamaguchi[1], Megumi Matsuo[1], Yasuharu Ueno[1], Shunsuke Tabe[1], Kenta Takahashi[1], Kenta Takeuchi[1], Eriko Kanai[1], Takashi Okumura[1], Toshiharu Kasai[1], and Hideki Taniguchi[1,2]:

[1] Division of Regenerative Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo

[2] Department of Regenerative Medicine, Graduate School of Medical Science, Yokohama City University

Early detection of pancreatic cancer is challenging due to the poor characterization of clinical symptoms. As pancreatic cancer is highly recurrent and high in metastasis rate, it is often resulting in a poor prognosis. Based on the organ bud technology developed in our regenerative medicine studies, we used primary pancreatic cancer cells isolated from Japanese pancreatic cancer patients to create a human primary pancreatic cancer organoid that could resemble the pancreatic cancer microenvironment. In this year, we modified further the pancreatic cancer organoids that we generated last year, and our current cancer organoid could closely recapitulatethe structure of clinical tissue in compared to those of conventional organoids.

In particular, our modified pancreatic cancer organoids possess various types of cancer-related fibroblasts (CAFs) which might contribute to the cancer malignancy. Moreover, modified organoids with a specific type of CAFs showed re-proliferation capacity of cancer cells after chemotherapy in vitro. To conclude, this novel cancer organoid has successfully improved in reproducing the characteristic of pancreatic cancer in vitro. Therefore, applying this new cancer organoid technology for therapeutic drug screening and biological analysis might play a vital role in contributing to the new findings.

5. Space Organogenesis (Development of advanced 3D organ culture system utilizing microgravity environment)

Tomomi Tadokoro[2], Yoshiki Kuse[1], Yasuharu Ueno[1], Takashi Okumura[1], Tomoyuki Yamaguchi[1] and Hideki Taniguchi[1,2]

[1] Division of Regenerative Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo

[2] Department of Regenerative Medicine, Graduate School of Medical Science, Yokohama City University

Microgravity in orbit does not cause subsidence or convection and is considered advantageous in expanding cells in three-dimension. By utilizing this microgravity environment, we aim to develop a novel method for generating human iPSCs-derived liver tissue in collaboration with Japan Aerospace Exploration Agency (JAXA). In detail, we attempt to establish the new technique of generating three-dimensional organs containing large blood vessels. We prepared human iPSCs derived liver buds (hiPSC-LBs) and artificial vessels on the earth. These were placed into the culture container and launched to the International Space Station "KIBO". From the first flight, we confirmed that hiPSC-LBs were successfully gathered onto the artificial vessel under microgravity, as *in silico* simulation suggested. After culturing hiPSC-LBs for a predetermined period in the incubator installed in "KIBO", samples were transferred to the earth. Adherence and fusion of hiPSC-LBs were observed on the artificial vessels in the samples cultured in orbit, especially endothelial cells started to extend their filopodia-like structure. Using qRT-PCR analysis of ground controls and flight samples, comparable expressions of hepatic, endothelial cell-related, and mesenchymal cell-related genes were observed in both samples. In addition, gene ontology analysis of RNA-seq data revealed that genes related to triglyceride homeostasis, cholesterol biosynthetic process, MAPK pathway, and angiogenesis were enriched in flight samples, indicating that space environment could provide optimal condition for tissue construction. These findings will uncover the effects of gravity on cell growth and differentiation. We expect the research results obtained in space experiment contribute to the subsequent development and understanding: (1) Development of a new technique for human three-dimensional tissue preservation and transportation, which is crucial to the product under regenerative medicine before practical use. (2) Establishment of a novel technique in generating human organs joined with large blood vessels. (3) Development of a new three-dimensional culture device simulating the microgravity environment on the earth.

6. Generation of bile duct tubules in hiPSC-liver buds

Erica Carolina[1], Ayumu Okumura[1], Yoshiki Kuse[1], Shinya Matsumoto[1], Takashi Okumura[1], Eriko Kanai[1], Toshiharu Kasai[1], Tomomi Tadokoro[2], Naoki

Tanimizu[1], and Hideki Taniguchi[1,2]
[1] Division of Regenerative Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo
[2] Department of Regenerative Medicine, Graduate School of Medical Science, Yokohama City University

Intrahepatic bile duct tubule (IHBD) is the crucial tissue structure for maintaining liver homeostasis by providing the excretion route for the bile secreted from hepatocytes. Although various types of liver organoids have been established, generation of tubular-shaped bile duct within hiPSC-liver organoid has not been reported. Here, we focus on an environmental cue to form tubular BDs, specifically the interaction between liver progenitors with portal vein (PV). To recapitulate fetal PV-BD tissue interaction, we developed a new co-culture system in which the hiPSC-liver progenitors are located next to the PV-like hiPSC-blood vessel (BV). Now we are refining the culture conditions to induce the differentiation of bipotential liver progenitor cells to cholangiocytes and the tubule formation of iPSC-BD in the three-dimensional co-culture system.

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Center for Stem Cell Biology and Regenerative Medicine

Division of Stem Cell and Molecular Medicine
幹細胞分子医学分野

Professor	Atsushi Iwama, M.D., Ph.D.	教　授	博士(医学)	岩　間　厚　志
Assistant Professor	Motohiko Oshima, Ph.D.	助　教	博士(医学)	大　島　基　彦
Assistant Professor	Yaeko Nakajima, Ph.D.	助　教	博士(医学)	中　島　やえ子
Assistant Professor	Masayuki Yamashita, M.D., Ph.D.	助　教	博士(医学)	山　下　真　幸
Project Assistant Professor	Kazumasa Aoyama, Ph.D.	特任助教	博士(医薬学)	青　山　和　正

Stem cells have the remarkable capacity to both self-renew and give rise to many types of more specialized cells in the body, which explains their great therapeutic potential in regenerative medicine. But that's not the only reason stem cells have become such a hotbed of scientific inquiry. These cellular transformers also offer an invaluable research tool for probing the disease mechanisms that underpin cancer, aging and a host of other health problems. Our major interest is to elucidate the mechanisms of self-renewal and multi-lineage differentiation of hematopoietic stem cells (HSCs). We are also interested in how the deregulated HSC functions are associated with aging of our body and the development of age-related hematological malignancies. We approach these issues mainly from the view point of epigenetics.

1. Limited rejuvenation of aged hematopoietic stem cells in young bone marrow niche

Wakako Kuribayashi[1,2], Motohiko Oshima[2], Naoki Itokawa[1,2], Shuhei Koide[2], Yaeko Nakajima-Takagi[2], Masayuki Yamashita[2], Satoshi Yamazaki[3,4], Bahityar Rahmutulla[5], Fumihito Miura[6], Takashi Ito[6], Atsushi Kaneda[5], and Atsushi Iwama[1,2] [1]Department of Cellular and Molecular Medicine, Graduate School of Medicine, Chiba University; [2]Division of Stem Cell and Molecular Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo; [3]Division of Stem Cell Biology, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, University of Tokyo; [4]Laboratory of Stem Cell Therapy, Faculty of Medicine, University of Tsukuba; [5]Department of Molecular Oncology, Graduate School of Medicine, Chiba University; [6]Department of Biochemistry, Kyushu University Graduate School of Medical Sciences.

Hematopoietic stem cells (HSCs) exhibit functional alterations, such as reduced regenerative capacity and myeloid-biased differentiation, with age. The HSC niche, which is essential for the maintenance of HSCs, also undergoes marked changes with aging. However, it has been technically challenging to directly evaluate the contribution of niche aging to age-associated HSC alterations without niche-damaging myeloablation in HSC transplantation assays. We herein transplanted an excess of aged HSCs into young mice without preconditioning. Although aged HSCs successfully engrafted in the intact young bone marrow niche, they poorly regenerated downstream progenitors and exhibited persistent myeloid-biased differentiation, resulting in no significant functional rejuvenation. Transcriptome and methylome analyses revealed that the young niche largely restored the transcriptional profile of aged HSCs, but not their DNA methylation profiles. Therefore, the restoration of the young niche is insufficient for rejuvenating HSC functions, highlighting a key role for age-associ-

ated cell-intrinsic defects in HSC aging.

2. PRC2 insufficiency causes p53-dependent dyserythropoiesis in myelodysplastic syndrome

Kazumasa Aoyama[1], Daisuke Shinoda[1,2], Emi Suzuki[1], Yaeko Nakajima-Takagi[1,2], Motohiko Oshima[1,2], Shuhei Koide[1,2], Ola Rizq[1,2], Sha Si[1], Shiro Tara[1], Goro Sashida[1,3], Atsushi Iwama[1,2] [1]Department of Cellular and Molecular Medicine, Graduate School of Medicine, Chiba University; [2]Division of Stem Cell and Molecular Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo; [3]Laboratory of Transcriptional Regulation in Leukemogenesis, International Research Center for Medical Sciences, Kumamoto University.

EZH1 and EZH2 are enzymatic components of polycomb repressive complex (PRC) 2, which catalyzes histone H3K27 tri- methylation (H3K27me3) to repress the transcription of PRC2 target genes. We previously reported that the hematopoietic cell-specific *Ezh2* deletion (*Ezh2*$^{\Delta/\Delta}$) induced a myelodysplastic syndrome (MDS)-like disease in mice. We herein demonstrated that severe PRC2 insufficiency induced by the deletion of one allele Ezh1 in Ezh2-deficient mice (*Ezh1*$^{+/-}$Ezh2$^{\Delta/\Delta}$) caused advanced dyserythropoiesis accompanied by a differentiation block and enhanced apoptosis in erythroblasts. p53, which is activated by impaired ribosome biogenesis in del(5q) MDS, was specifically activated in erythroblasts, but not in hematopoietic stem or progenitor cells in *Ezh1*$^{+/-}$ Ezh2$^{\Delta/\Delta}$ mice. *Cdkn2a*, a major PRC2 target encoding *p19*^{Arf,} which activates p53 by inhibiting MDM2 E3 ubiquitin ligase, was de-repressed in *Ezh1*$^{+/-}$Ezh2$^{\Delta/\Delta}$ erythroblasts. The deletion of *Cdkn2a* as well as p53 rescued dyserythropoiesis in *Ezh1*$^{+/-}$Ezh2$^{\Delta/\Delta}$ mice, indicating that PRC2 insufficiency caused p53-dependent dyserythropoiesis via the de-repression of *Cdkn2a*. Since PRC2 insufficiency is often involved in the pathogenesis of MDS, the present results suggest that p53-dependent dyserythropoiesis manifests in MDS in the setting of PRC2 insufficiency.

3. DHODH inhibition synergizes with DNA-demethylating agents in the treatment of myelodysplastic syndromes

Kensuke Kayamori[1,2], Yurie Nagai[2], Cheng Zhong[1], Satoshi Kaito[1], Daisuke Shinoda[1], Shuhei Koide[1], Wakako Kuribayashi[1], Motohiko Oshima[1], Yaeko Nakajima-Takagi[1], Masayuki Yamashita[1], Naoya Mimura[3], Hans Jiro Becker[4], Kiyoko Izawa[4], Satoshi Yamazaki[4,5], Satoshi Iwano[6], Atsushi Miyawaki[6], Ryoji Ito[7], Kaoru Tohyama[8], William Lennox[9], Josephine Sheedy[9], Marla Weetall[9], Emiko Sakaida[2], Koutaro Yokote[2], and Atsushi Iwama[1] [1]Division of Stem Cell and Molecular Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo; [2]Department of Endocrinology, Hematology and Gerontology, Chiba University Graduate School of Medicine; 3Department of Transfusion Medicine and Cell Therapy, Chiba University Hospital; [4]Division of Stem Cell Biology, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo; [5]Laboratory of Stem Cell Therapy, Faculty of Medicine, University of Tsukuba; [6]Laboratory for Cell Function Dynamics, Center for Brain Science, RIKEN; [7]Central Institute for Experimental Animals; [8]Department of Laboratory Medicine, Kawasaki Medical School; and [9]PTC Therapeutics Inc

Dihydroorotate dehydrogenase (DHODH) catalyzes a rate-limiting step in de novo pyrimidine nucleotide synthesis. DHODH inhibition has recently been recognized as a potential new approach for treating acute myeloid leukemia (AML) by inducing differentiation. We investigated the efficacy of PTC299, a novel DHODH inhibitor, for myelodysplastic syndrome (MDS). PTC299 inhibited the proliferation of MDS cell lines, and this was rescued by exogenous uridine, which bypasses de novo pyrimidine synthesis. In contrast to AML cells, PTC299 was inefficient at inhibiting growth and inducing the differentiation of MDS cells, but synergized with hypomethylating agents, such as decitabine, to inhibit the growth of MDS cells. This synergistic effect was confirmed in primary MDS samples. As a single agent, PTC299 prolonged the survival of mice in xenograft models using MDS cell lines, and was more potent in combination with decitabine. Mechanistically, a treatment with PTC299 induced intra-S-phase arrest followed by apoptotic cell death. Of interest, PTC299 enhanced the incorporation of decitabine, an analog of cytidine, into DNA by inhibiting pyrimidine production, thereby enhancing the cytotoxic effects of decitabine. RNA-seq data revealed the marked downregulation of MYC target gene sets with PTC299 exposure. Transfection of MDS cell lines with MYC largely attenuated the growth inhibitory effects of PTC299, suggesting MYC as one of the major targets of PTC299. Our results indicate that the DHODH inhibitor PTC299 suppresses the growth of MDS cells and acts in a synergistic manner with decitabine. This combination therapy may be a new therapeutic option for the treatment of MDS.

4. Insufficiency of non-canonical PRC1 synergizes with JAK2V617F in the development of myelofibrosis

Daisuke Shinoda[1,2], Yaeko Nakajima-Takagi[1,2], Motohiko Oshima[1,2], Shuhei Koide[1,2], Kazumasa Aoyama[2], Atsunori Saraya[2], Hironori Harada[3], Bah-

ityar Rahmutulla[4], Atsushi Kaneda[4], Kiyoshi Yamaguchi[5], Yoichi Furukawa[5], Haruhiko Koseki[6], Kazuya Shimoda[7], Tomoaki Tanaka[8], Goro Sashida[9] and Atsushi Iwama[1,2,10] [1]Division of Stem Cell and Molecular Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo; [2]Department of Cellular and Molecular Medicine, Graduate School of Medicine, Chiba University; [3]Laboratory of Oncology, Tokyo University of Pharmacy and Life Sciences; [4]Department of Molecular Oncology, Graduate School of Medicine, Chiba University; [5]Division of Clinical Genome Research, Advanced Clinical Research Center, The Institute of Medical Science, The University of Tokyo; [6]Laboratory for Developmental Genetics, RIKEN Center for Integrative Medical Sciences; [7]Division of Hematology, Diabetes, and Endocrinology, Department of Internal Medicine, Faculty of Medicine, University of Miyazaki; [8]Department of Molecular Diagnosis, Chiba University Graduate School of Medicine; [9]Laboratory of Transcriptional Regulation in Leukemogenesis, International Research Center for Medical Sciences, Kumamoto University; [10]Laboratoty of Cellular and Molecular Chemistry, Graduate School of Pharmaceutical Sciences, The University of Tokyo.

Insufficiency of polycomb repressive complex 2 (PRC2), which trimethylates histone H3 at lysine 27, is frequently found in primary myelofibrosis and promotes the development of JAK2V617F-induced myelofibrosis in mice by enhancing the production of dysplastic megakaryocytes. Polycomb group ring finger protein 1 (Pcgf1) is a component of PRC1.1, a non-canonical PRC1 that monoubiquitylates H2A at lysine 119 (H2AK119ub1). We herein investigated the impact of PRC1.1 insufficiency on myelofibrosis. The deletion of Pcgf1 in JAK2V617F mice strongly promoted the development of lethal myelofibrosis accompanied by a block in erythroid differentiation. Transcriptome and chromatin immunoprecipitation sequence analyses showed the de-repression of PRC1.1 target genes in Pcgf1-de!cient JAK2V617F hematopoietic progenitors and revealed Hoxa cluster genes as direct targets. The deletion of Pcgf1 in JAK2V617F hematopoietic stem and progenitor cells (HSPCs), as well as the overexpression of Hoxa9, restored the attenuated proliferation of JAK2V617F progenitors. The overexpression of Hoxa9 also enhanced JAK2V617F-mediated myelofibrosis. The expression of PRC2 target genes identified in PRC2-insufficient JAK2V617F HSPCs was not largely altered in Pcgf1-deleted JAK2V617F HSPCs. The present results revealed a tumor suppressor function for PRC1.1 in myelofibrosis and suggest that PRC1.1 insufficiency has a different impact from that of PRC2 insufficiency on the pathogenesis of myelofibrosis.

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Center for Stem Cell Biology and Regenerative Medicine

Division of Stem Cell Transplantation
幹細胞移植分野

Professor	Yasuhito Nannya, M.D., D.M.Sc.	教　授	博士(医学)　南　谷　泰　仁
Project Professor	Satoshi Takahashi, M.D., D.M.Sc.	特任教授	博士(医学)　高　橋　　聡

We are researching the clinical promotion and medical development of hematopoietic stem cell transplantation, with a focus on cord blood transplantation. We are also working on the identification of factors involved in transplant complications using GWAS, with the aim of making transplantation safer. We are also generating pre-clinical study to utilize virus-specific CTL for immune competent patients such as post-transplantation. Our goal is as allogeneic transplantation to be safer therapeutic option and to extend for older patients.

1. Long-term outcomes following the addition of granulocyte colony-stimulating factor-combined high-dose cytarabine to total body irradiation and cyclophosphamide conditioning in single-unit cord blood transplantation for myeloid malignancies

Konuma, T[1], Kato, S[1], Isobe[1], M. Monna-Oiwa[1], M. Tojo, A[1]. Iseki, T. Takahashi, S[1,3].

[1] Division of Hematopoietic Disease Control

[2] Department of Hematology/Oncology, IMSUT Hospital

[3] Division of Stem Cell Transplantation

An intensified myeloablative conditioning regimen, involving the addition of granulocyte colony-stimulating factor (G-CSF)-combined high-dose cytarabine (12 g/m(2)) to standard total body irradiation and cyclophosphamide, has been performed for adult patients with myeloid malignancies in single-unit cord blood transplantation (CBT) since 1998 in our institute. We update the results of CBT, as the first allogeneic hematopoietic cell transplantation after this conditioning regimen, in 169 patients with a median long-term follow-up of 10.4 years. The median age was 43 years (range, 16 to 59 years). Ninety-four patients (56%) were in non-remission at the time of CBT, and 124 patients (73%) were acute myeloid leukemia. The median cryopreserved cord blood total nucleated cell dose and CD34(+) cell dose was 2.40 x 10(7)/kg and 0.93 x 10(5)/kg, respectively. The cumulative incidence of neutrophil recovery at 42 days was 94.4% (95% confidence interval [CI]: 88.6-97.3%). Among the whole cohort, 105 patients were still alive at the end of the study period. The cumulative incidences of relapse and non-relapse mortality at 10 years were 26.0% (95% CI: 19.5-33.0%) and 16.9% (95% CI: 11.4-23.4%), respectively. There was an overall survival probability of 62.5% (95% CI: 54.3-69.7%) at 10 years. Higher disease risk index alone significantly affected higher overall mortality (hazard ratio 2.21, P = 0.003) in multivariate analysis. These outcomes demonstrate that G-CSF-combined myeloablative conditioning could have favorable long-term remission rates for adult patients with myeloid malignancies undergoing single-unit CBT.

2. Early-Phase Peripheral Blood Eosinophilia Predicts Lower Overall and Non-Relapse Mortality After Single-Unit Cord Blood Transplantation.

Konuma, T[1,2], Monna-Oiwa, M[2], Kaito, Y[1], Isobe, M[1], Okabe, M[1], Kato, S.[1], Takahashi, S[1,3].
Tojo, A[2,4].

1 Division of Hematopoietic Disease Control
2 Department of Hematology/Oncology, IMSUT Hospital
3 Division of Stem Cell Transplantation
4 Division of Molecular Therapy

Peripheral blood eosinophilia has been associated with the development of graft-versus-host disease (GVHD) and survival after allogeneic hematopoietic cell transplantation (HCT). However, the impacts of eosinophilia on cord blood transplantation (CBT) outcomes remain unclear. The objective of this study was to examine the associations between eosinophilia and overall survival, relapse incidence, non-relapse mortality, and acute and chronic GVHD after single-unit CBT for adults. We retrospectively analyzed the data for 225 adult patients who received single-unit CBT at our institute between March 2004 and March 2020. The cumulative incidence of eosinophilia, defined as an absolute eosinophil count of >/=500 x 10(6)/L in peripheral blood, was 48.9% (95% confidence interval, 42.2% to 55.2%) at 60 days after CBT. Recipient cytomegalovirus seronegative status and higher cryopreserved cord blood CD34(+) cell dose were significantly associated with a higher incidence of eosinophilia after CBT. Among patients who achieved neutrophil recovery, neutrophil recovery was significantly earlier in patient with eosinophilia compared to those without eosinophilia (P = .016). Serum levels of interleukin-5 at 4 weeks were significantly higher in patients with eosinophilia compared with those without eosinophilia (P = .041). Multivariate analysis, in which the development of eosinophilia was treated as a time-dependent covariate, showed that eosinophilia was significantly associated with lower overall mortality (hazard ratio [HR], .58; P = .034) and non-relapse mortality (HR, .41; P = .029), but not relapse incidence or development of acute or chronic GVHD. Our data suggested that early-phase eosinophilia is a predictor of favorable outcomes in adult patients undergoing single-unit CBT.

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Center for Stem Cell Biology and Regenerative Medicine

Division of Stem Cell Signaling
幹細胞シグナル制御分野

Professor Toshio Kitamura, M.D., D.M.Sc.

教授 医学博士 北 村 俊 雄

Our major interest is to elucidate the mechanisms of pluripotency, self-renewal and the control of cell division and differentiation of hematopoietic stem and progenitor cells. We have developed the retrovirus-mediated efficient gene transfer and several functional expression cloning systems, and utilized these system to our experiments. We are now conducting several projects related to stem cells to characterize stem cells, clarify underling mechanisms of maintenance of pluripotency, and differentiation.

1. Developing Analysis Tools for Cell Cycle and Cell Division of Hematopoietic Stem Cells: MgcRacGap-hmKusabiraOrange2 (MRG-hmKuO2) fusion protein for midbody marker.

Yosuke Tanaka, Tsuyoshi Fukushima, Toshihiko Oki, Kotarou Nishimura, Asako Sakaue-Sawano[1], Atsushi Miyawaki[1], Toshio Kitamura: [1]Laboratory for Cell Function Dynamics, RIKEN, Wako, Saitama and ERATO Miyawaki Life Function Dynamics Project, JST.

Previously, we reported that MgcRacGap is a marker for midbody and that MgcRacGap-mVenus fusion protein visualized asymmetric inheritance and release of midbody during cytokinesis (Nishimura et al., 2013). We retrovirally introduced MRG-hmKuO2 into hematopoietic stem cells (HSCs), in order to examine whether midbody asymmetric inheritance and release is involved with asymmetric division of HSCs. HSCs showed high frequency of midbody release during cytokinesis in culture. Interestingly, one daughter cell releasing midbody differentiated earlier than the other daughter cell inheriting midbody. We generated Cre-inducible MRG-hmKuO2 mouse line. Briefly, the MRG-hmKuO2 fusion gene is inserted into Rosa26 locus following a loxP-NEO-STOP-loxP cassette, in order to visualize asymmetric inheritance and release of midbody in vivo without retroviral infection. Crossing MRG-hmKuO2 mice with Vav-Cre mice, MRG-hmKuO2 nicely marked midbody asymmetric inheritance and release in HSCs in culture. We are planning to do paired-daughter assay using HSCs from MRG-hmKuO2 mice to examine whether inheritance and release of midbody link to asymmetric division of HSCs. Given that some problems were found in this new mouse line, we are planning to establish surrogate experimental models for this.

Next we performed long-term, live single-cell imaging and tracking during HSC division with future cell-fate quantification and MRG inheritance of daughter cells. We expressed MRG-hmKuO2 in mouse HSCs, and quantified their inheritance during HSC division in vitro. We observed the linkage between midbody release and stem/progenitor cell potential. That was more obvious after 1st division and the tendency was decreased by increasing dvision times. We also counted lineage marker positive cells 4 days after culture. The detection antibodies (CD16/32 for myeloid cells and CD71 for erythroid progenitor cells) were put in culture at the beginning of the culture. Lineage restriction was not linked to the inheritance of midbody. These results indicated that midbody release would be one of features of keeping stemness in HSC asymmetric division and dose not affect lineage commitment.

2. Developing Analysis Tools for Cell Cycle and Cell Division of Hematopoietic Stem Cells and Leukemic Stem Cells: A novel G_0 marker (G_0M), mVenus-p27K- and its transgenic mouse

Tsuyoshi Fukushima, Yosuke Tanaka, Toshihiko Oki, Kotarou Nishimura, Keito Adachi, Keiko Mikami, Asako Sakaue-Sawano[1], Atsushi Miyawaki[1], Toshio Kitamura: [1]Laboratory for Cell Function Dynamics, RIKEN, Wako, Saitama and ERATO Miyawaki Life Function Dynamics Project, JST.

One of the common features of the stem cells is that they are in quiescent (G_0) phase of cell cycle. Several reports indicate that tissue specific stem cells like hematopoietic stem cells (HSCs) and cancer stem cells are in G_0 phase.

We have developed a novel G_0 marker (G_0M), mVenus-p27K- (Oki et al, 2013). The G_0M clearly marked the cells in G_0 and very early G_1 in NIH3T3 cells. To examine G_0 status in HSCs, we generated a G_0M mouse line where hamtopoietic cells express mVenus-p27K- fusion gene. Interestingly, three different fractions (G_0M-high (70%), G_0M-low (20%), G_0M-negative (10%)) were identified in the HSC fraction (CD150+CD48-cKit+Sca-1+Lineage-). G_0M-high/low fractions but not G_0M-negative fraction showed an ability to reconstitute multi-lineage blood cells. BrdU-label retaining assay, a method for detection of dormant cells in various tissues, showed that G_0M-high population contained dormant functional HSCs and G_0M-low population contained active functional HSCs. Single-cell RNA sequence (scRNA-seq) analysis showed that G_0M-high cells expressed well-known HSC-related genes including *Hlf*, *Ifitm1*, *Mpl* and *Ly6a*. On the other hand, highly expressed genes in G_0M-low cells included genes associated with cell cycle or differentiation, such as *Gata1*, *Itga2b* and *Cdk6*. Small-cell Mass Spec analysis showed that Cdk6 protein was detected in G_0M-low fraction, but not in G_0M-high fraction. Taken together, these data exhibited that G_0M could discriminate dormant and active functional HSCs in the conventional HSC fraction. Moreover, high-throughput small molecule screening revealed that high concentrations of cytoplasmic calcium ($[Ca^{2+}]_c$) were linked to dormancy of HSCs. Of note, upregulation $[Ca^{2+}]_c$ by thapsigargin, a sarco/endoplasmic reticulum calcium-ATPase (SERCA) inhibitor, which increases $[Ca^{2+}]_c$ by leaking calcium from ER, could enhanced bone marrow multi-lineage reconstitution ability of LT-HSCs. These findings indicate that G_0M separates dormant and active adult HSCs, which are regulated by Cdk4/6 and $[Ca^{2+}]_c$.

To get more insight about the regulaton of stemness, we performed RNA-Seq analysis between dormant and active adult HSCs and found that their gene expression differecies were small. This result encouraged us to assess their enhancer expression differencies, as gene expressions are regulated by enhancers in general. We identified about 400 enhancers highly expressed in doramnt HSCs. Using public ChipSeq data of histons and HSC-specific transcription factors, we identified about 100 dormant HSC-specific enhancers. Now we are planning to further identify functional enhancers by manupurating those enhancers in HSCs.

3. CRISPR/Cas9-mediated base-editing enables a chain reaction through sequential repair of sgRNA scaffold mutations

Tsuyoshi Fukushima, Yosuke Tanaka, Keito Adachi, Nanami Masuyama[1,2], Akiho Tsuchiya, Shuhei Asada, Soh Ishiguro[1,2], Hideto Mori[1], Motoaki Seki[1], Nozomu Yachie[1,2], Susumu Goyama, Toshio Kitamura
[1]Research Center for Advanced Science and Technology, The University of Tokyo, Tokyo 153-8904, Japan
[2]School of Biomedical Engineering, The University of British Columbia, Vancouver, BC V6T 1Z3, Canada

Cell behavior is controlled by complex gene regulatory networks. Although studies have uncovered diverse roles of individual genes, it has been challenging to record or control sequential genetic events in living cells. In this study, we designed two cellular chain reaction systems that enable sequential sgRNA activation in mammalian cells using a nickase Cas9 tethering of a cytosine nucleotide deaminase (nCas9-CDA). In these systems, thymidine (T)-to-cytosine (C) substitutions in the scaffold region of the sgRNA or the TATA box-containing loxP sequence (TATAloxP) are corrected by the nCas9-CDA, leading to activation of the next sgRNA. These reactions can occur multiple times, resulting in cellular chain reactions. As a proof of concept, we established a chain reaction by repairing sgRNA scaffold mutations in 293T cells. Importantly, the results obtained in yeast or in vitro did not match those obtained in mammalian cells, suggesting that in vivo chain reactions need to be optimized in appropriate cellular contexts. Our system may lay the foundation for building cellular chain reaction systems that have a broad utility in the future biomedical research.

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Center for Stem Cell Biology and Regenerative Medicine

Division of Stem Cell Processing
幹細胞プロセシング分野

Professor Hideki Taniguchi, M.D., Ph.D.

教 授 博士(医学) 谷 口 英 樹

Given their unique regenerative abilities and scarcity, stem cell is a valuable cell source in the field of regenerative medicine. As representative example, the transplantation of a small number of hematopoietic stem cells (HSCs) which can give rise into all types of blood cells is now widely used to treat patients who have lost their natural ability to produce blood due to leukemia or genetic diseases. Further development of research by using pluripotent stem cells are newly emerging types of stem cells that have been utilized either for the basic research or to develop curative treatment for various diseases. We have been focusing especially on the utilization of induced human pluripotent stem cells (hiPSCs) as a research platform to elucidate pathophysiology of intractable diseases based on their proper modeling. Our goal is to establish a safe and efficacious treatment for the patients suffering from incurable diseases.

Establishment of high-throughput screening platform for RAS-associated autoimmune lymphoproliferative syndrome-like disorder (RALD)

Yun-Zhong NIE[1], Yang LI[1], Tomoyuki Yamaguchi[1], Takashi Okumura[1], Yumi Horie[1], Huan-Ting Lin[1], Hideki Taniguchi[1,2]

[1] Division of Regenerative Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo

[2] Department of Regenerative Medicine, Graduate School of Medical Science, Yokohama City University

RAS-associated autoimmune lymphoproliferative syndrome-like disorder (RALD) is a rare genetic chronic disorder of the immune system, characterized by persistent monocytosis and is often associated with leukocytosis, lymphoproliferation, and autoimmune phenomena. RALD is caused by gain-of-function somatic mutations in the genes NRAS or KRAS, which present in the hematopoietic lineages, but not in other somatic cells, suggesting RALD is a somatic disorder arising in an early precursor or hematopoietic stem cell. We previously generated human induced pluripotent stem cells (iPSCs) with $KRAS^{G13C}$ mutation from hematopoietic lineages of RALD patients. iPSC-derived hematopoietic progenitor cells from patients could reproduce the abnormal hematopoiesis in vitro and could be used to screen drugs targeting $KRAS^{G13C}$ mutation. Our data revealed that human iPSC-derived hematopoietic progenitor cell is an effective model screening candidate for RALD treatment. Besides $KRAS^{G13C}$ mutation, we also try to establish the screening platform for other KRAS or NRAS mutation types in RALD. To expand a series of RALD-related iPSCs, we perform genome-editing technologies to induce mutations in the KRAS or NRAS genes. Moreover, we develop a feeder-free protocol to differentiate hematopoietic progenitor cells from human iPSCs. The purity of hematopoietic progenitor cells generated by this protocol is as high as 90%, and the cell number is ten times that of the previous protocol, making it possible to establish a high-throughput screening platform for RALD drug screening. By the combination of both genome-editing and iPSC differentiation technologies, we hope to develop ideal treatment strategies for RAS-associated intractable disorders with a high-throughput screening platform.

Center for Stem Cell Biology and Regenerative Medicine

Division of Experimental Pathology
幹細胞病理学分野

Professor Yasuhiro Yamada M.D., Ph.D.

教 授 博士(医学) 山 田 泰 広

Stem cells play an important role in homeostasis of organ function in multicellular organisms. They are responsible for tissue regeneration and their functional impairment causes various diseases in mammals. However, considering the complexity of multicellular organisms, it remains unclear how tissue microenvironment affects stem cell functions. We aim to elucidate the molecular basis for stem cell behavior in response to altered tissue microenvironments. The effort should eventually unveil the fundamental basis of how stem cells affect organismal functions in vivo and uncover the underlying mechanisms of tissue regeneration, various diseases and organismal aging. These findings may contribute to developing a feasible strategy to control the detrimental effects of stem cell dysfunction in diseases and aging.

1. Generation of mice for evaluating endogenous p16^{Ink4a} protein expression

Yui Shimada-Takayama, Takayuki Yasuda, Tomoyo Ukai, Jumpei Taguchi, Manabu Ozawa[1], Nao Sankoda, Sho Ohta, Yasuhiro Yamada[2]

[1] Laboratory of Reproductive Systems Biology, Institute of Medical Science, University of Tokyo

[2] AMED-CREST, AMED.

The cyclin-dependent kinase inhibitor p16^{Ink4a} plays a central role in cellular senescence *in vitro*. Although previous studies suggested cellular senescence is integrated in the systemic mechanisms of organismal aging, the localization and the dynamics of p16^{Ink4a} in tissues remain poorly understood, which hinders uncovering the role of p16^{Ink4a} under the *in vivo* context. One of the reasons is due to the lack of reliable reagents; as we also demonstrate here that commonly used antibodies raised against human p16^{Ink4a} barely recognize its murine ortholog. Here we generated a mouse model, in which the endogenous p16^{Ink4a} is HA-tagged at its N-terminus, to explore the protein expression of p16^{Ink4a} at the organismal level. p16^{Ink4a} was induced at the protein level along the course of senescence in primary embryonic fibroblasts derived from the mice, consistently to its transcriptional level. Remarkably, however, p16^{Ink4a} was not detected in the tissues of the mice exposed to pro-senescence conditions including genotoxic stress and activation of oncogenic signaling pathways, indicating that there is only subtle p16^{Ink4a} proteins induced. These results in our mouse model highlight the need for caution in evaluating p16^{Ink4a} protein expression *in vivo*.

2. DMRT1-mediated *in vivo* reprogramming drives development of cancer resembling human germ cell tumors with features of totipotency

Jumpei Taguchi, Hirofumi Shibata[3,4], Mio Kabata[3], Masaki Kato[5,6], Kei Fukuda[5], Akito Tanaka[3], Sho Ohta, Tomoyo Ukai, Kanae Mitsunaga[3], Yosuke Yamada[3,7], So Nagaoka[8,9], Sho Yamazawa[10], Kotaro Ohnishi[3,11], Knut Woltjen[3], Tetsuo Ushiku[10], Manabu Ozawa[1], Mitinori Saitou[3,8,12], Yoichi Shinkai[5], Takuya Yamamoto[3,12,13,2] and Yasuhiro Yamada[2]

[3]Department of Life Science Frontiers, Center for iPS Cell Research and Application (CiRA), Kyoto

University.
[4]Department of Otolaryngology, Gifu University Graduate School of Medicine.
[5]Cellular Memory Laboratory, RIKEN Cluster for Pioneering Research.
[6]Laboratory for Transcriptome Technology, RIKEN Center for Integrative Medical Sciences.
[7]Department of Diagnostic Pathology, Kyoto University Hospital.
[8]Department of Anatomy and Cell Biology, Graduate School of Medicine, Kyoto University.
[9]Department of Embryology, Nara Medical University.
[10]Department of Pathology, Graduate School of Medicine, The University of Tokyo.
[11]Department of Gastroenterology/Internal Medicine, Gifu University Graduate School of Medicine.
[12]Institute for the Advanced Study of Human Biology (WPI-ASHBi), Kyoto University.
[13]Medical-risk Avoidance Based on iPS Cells Team, RIKEN Center for Advanced Intelligence Project (AIP).

In vivo reprogramming provokes a wide range of cell fate conversion. We found that *in vivo* induction of higher levels of OSKM in mouse somatic cells leads to increased expression of primordial germ cell (PGC)-related genes and provokes genome-wide erasure of genomic imprinting, which takes place exclusively in PGCs. *In vivo* OSKM reprogramming caused propagation of OCT4/NANOG-positive cells, resulting in development of cancer that resembled human germ cell tumors. Like a subgroup of germ cell tumors, propagated tumor cells could differentiate into trophoblasts. Moreover, these tumor cells gave rise to induced pluripotent stem cells (iPSCs) with expanded differentiation potential that could contribute to adult mice. DMRT1, which is expressed in PGCs, drove the reprogramming and propagation of the tumor cells *in vivo*. Furthermore, DMRT1-mediated reprogramming is associated with trophoblast competence of the reprogrammed cells and provides a therapeutic target for germ cell tumors. These results reveal a novel route for somatic cell reprogramming and underscore the impact of reprogramming in development of germ cell tumors. Furthermore, our findings may have implications regarding acquisition of totipotency-like features by somatic cells.

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Center for Stem Cell Biology and Regenerative Medicine

Division of Stem Cell Biology
幹細胞生物学分野

Project Associate Professor Satoshi Yamazaki, Ph.D. | 特任准教授 博士(生命科学) 山 﨑 聡

Our studies focus mainly on investigation of stem cell biology using the hematopoietic stem cell (HSC) as a research model. Recent identification of a variety of stem cell sources including embryonic and somatic (tissue-specific) stem cells has brought about substantial progress in the field of stem cell research.

1. Polyvinyl alcohol hydrolysis rate and molecular weight influence human and murine HSC activity ex vivo.

Kazuhiro Sudo [1], Satoshi Yamazaki [2], Adam C Wilkinson [3], Hiromitsu Nakauchi [4], Yukio Nakamura [5]
[1]Cell Engineering Division, BioResource Research Center, RIKEN, Tsukuba, Japan.
[2]Division of Stem Cell Therapy, Distinguished Professor Unit, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan; Laboratory of Stem Cell Therapy, Faculty of Medicine, University of Tsukuba, Ibaraki, Japan.
[3]MRC Molecular Haematology Unit, MRC Weatherall Institute of Molecular Medicine, University of Oxford, UK.
[4]Division of Stem Cell Therapy, Distinguished Professor Unit, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan; Institute for Stem Cell Biology and Regenerative Medicine and Department of Genetics, Stanford University School of Medicine, Stanford, CA, USA.
[5]Cell Engineering Division, BioResource Research Center, RIKEN, Tsukuba, Japan.

Ex vivo expansion of hematopoietic stem cells (HSCs) is one of the most promising strategies to increase the availability of transplantable HSCs and improve bone marrow transplantation outcomes. We recently demonstrated that mouse HSCs could be efficiently expanded in polyvinyl alcohol (PVA)-containing culture medium using only recombinant stem cell factor and thrombopoietin cytokines. However, the behavior of human HSCs in these simple PVA-based media was not fully elucidated. In this study, we analyzed the compatibility of PVA of different hydrolysis rates (HR) and molecular weights (MW) to support functional human and mouse HSCs ex vivo. Human and mouse HSCs proliferated more frequently in media containing PVA with lower HR than with higher HR, but both PVA types supported HSC multilineage reconstitution potential. Importantly, human HSCs cultured in PVA-containing media engrafted not only in irradiated recipients but also in non-irradiated recipients. Our results demonstrate that human HSCs can be maintained ex vivo using PVA-based culture systems and suggest approaches for future optimization of human HSC expansion.

2. Non-conditioned bone marrow chimeric mouse generation using culture-based enrichment of hematopoietic stem and progenitor cells.

Kiyosumi Ochi [#1], Maiko Morita [#1], Adam C Wilkinson [2], Atsushi Iwama [3], Satoshi Yamazaki [4 5]
[1]Division of Stem Cell Biology, Center for Stem Cell Biology and Regenerative Medicine, The Institute of

Medical Science, The University of Tokyo, Tokyo, Japan.
[2]MRC Molecular Hematology Unit, MRC Weatherall Institute of Molecular Medicine, University of Oxford, Radcliffe Department of Medicine, University of Oxford, Oxford, UK.
[3]Division of Stem Cell and Molecular Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.
[4]Division of Stem Cell Biology, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.
[5]Laboratory of Stem Cell Therapy, Faculty of Medicine, University of Tsukuba, Ibaraki, Japan.
[#]Contributed equally.

Bone marrow (BM) chimeric mice are a valuable tool in the field of immunology, with the genetic manipulation of donor cells widely used to study gene function under physiological and pathological settings. To date, however, BM chimera protocols require myeloablative conditioning of recipient mice, which dramatically alters steady-state hematopoiesis. Additionally, most protocols use fluorescence-activated cell sorting (FACS) of hematopoietic stem/progenitor cells (HSPCs) for ex vivo genetic manipulation. Here, we describe our development of cell culture techniques for the enrichment of functional HSPCs from mouse BM without the use of FACS purification. Furthermore, the large number of HSPCs derived from these cultures generate BM chimeric mice without irradiation. These HSPC cultures can also be genetically manipulated by viral transduction, to allow for doxycycline-inducible transgene expression in donor-derived immune cells within non-conditioned immunocompetent recipients. This technique is therefore expected to overcome current limitations in mouse transplantation models.

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Center for Stem Cell Biology and Regenerative Medicine

Division of Mammalian Embryology
再生発生学分野

Project Associate Professor Toshihiro Kobayashi, Ph.D. ｜ 特任准教授 博士(生命科学) 小 林 俊 寛

The aim of our lab is to understand how the pluripotent cells decide the fate in early mammalian embryos and to apply their principle for future regenerative medicine. In particular, we use pluripotent stem cells and early embryos from various mammals, not only mice and humans. This approach enables us to investigate conserved mechanisms among the mammals and to develop novel technology by the use of species-specific features.

1. Tracing the emergence of primordial germ cells from bilaminar disc rabbit embryos and pluripotent stem cells

Toshihiro Kobayashi[1,2], Aracely Castillo-Venzor[3,4,5], Chris A. Penfold[4], Michael Morgan[6,7], Naoaki Mizuno[8], Walfred W.C. Tang[3,4], Yasuyuki Osada[9], Masao Hirao[9], Fumika Yoshida[2], Hideyuki Sato[8], Hiromitsu Nakauchi[8,10], Masumi Hirabayashi[2,11], M. Azim Surani[3,4]

1 Division of Mammalian Embryology, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo, 2 Center for Genetic Analysis of Behavior, National Institute for Physiological Sciences, 3 Wellcome Trust/Cancer Research UK Gurdon Institute, University of Cambridge, 4 Department of Physiology, Development and Neuroscience, University of Cambridge, 5 Wellcome Trust–Medical Research Council Cambridge Stem Cell Institute, Jeffrey Cheah Biomedical Centre, University of Cambridge, 6 Cancer Research UK Cambridge Institute, University of Cambridge, 7 European Molecular Biology Laboratory, European Bioinformatics Institute, 8 Division of Stem Cell Therapy, Distinguished Professor Unit, The Institute of Medical Science, The University of Tokyo, 9 Ina Bio Center, Kitayama Labes Co., Ltd., 10 Institute for Stem Cell Biology and Regenerative Medicine, Department of Genetics, Stanford University School of Medicine, 11 The Graduate University of Advanced Studies

Rabbit embryos develop as bilaminar discs at gastrulation as in humans and most other mammals, whereas rodents develop as egg cylinders. Primordial germ cells (PGCs), founder cells of sperm and egg, appear to originate during gastrulation according to many systematic studies on mammalian embryos. In this project, we showed that rabbit PGC (rbPGC) specification occurs at the posterior epiblast at the onset of gastrulation. Using newly derived rabbit pluripotent stem cells, we showed robust and rapid induction of rbPGC-like cells *in vitro* with WNT and BMP morphogens, which reveals *SOX17* as the critical regulator of rbPGC fate as in several non-rodent mammals such as human and pig. Notably, single cell RNA-sequencing analysis revealed that the transcriptome of rbPGC-like cells is highly similar to that of *in vivo* nascent rbPGCs. We posit that development as a bilaminar disc is a crucial determinant of the PGC regulators, regardless of the highly diverse development of extraembryonic tissues, including the amnion. We propose that investigations on rabbits with short gestation, large litters, and where gastrulation precedes implantation can contribute significantly to advances in early mammalian development.

2. Pluripotent stem cells corresponding to embryonic disc exhibit common self-renewal requirements in diverse livestock species

Masaki Kinoshita[12], Toshihiro Kobayashi, Benjamin Planells[13], Doris Klisch[13], Daniel Spindlow[12,14], Hideki Masaki, Susanne Bornelöv[12], Giuliano Giuseppe Stirparo[12,14], Hitomi Matsunari[15], Ayuko Uchikura[15], Ismael Lamas-Toranzo[12,14], Jennifer Nichols[12,16], Hiromitsu Nakauchi, Hiroshi Nagashima[15], Ramiro Alberio[13], Austin Smith[12,14]

12 Wellcome-MRC Cambridge Stem Cell Institute, Jeffery Cheah Biomedical Centre, University of Cambridge, 13 School of Biosciences, University of Nottingham, 14 Living Systems Institute, University of Exeter,15 Laboratory of Medical Bioengineering, Department of Life Sciences, School of Agriculture, Meiji University, 16 Department of Physiology, Development and Neuroscience, University of Cambridge,

Despite four decades of effort, robust propagation of pluripotent stem cells from livestock animals remains challenging. The requirements for self-renewal are unclear and the relationship of cultured stem cells to pluripotent cells resident in the embryo uncertain. In this project, we applied culture condition developed for supporting robust sefl-renewal and expansion of undifferentiated rabbit pluripotent stem cells to livestock's. Also, we avoided feeder cells or serum factors to provide a defined culture microenvironment. We show that the combination of Activin A, Fibroblast growth factor, and Wnt inhibitor XAV939 (AFX), supported establishment and continuous expansion of pluripotent stem cell lines from porcine, ovine and bovine embryos. Germ layer differentiation was evident in teratomas and could be induced *in vitro*. Global transcriptome analyses highlighted commonality across the three species, while comparison with porcine embryo stages showed proximity to bilaminar disc epiblast. Clonal genetic manipulation and gene targeting were exemplified in porcine stem cells. Finally, we demonstrated that genetically modified AFX stem cells which was introduced T2A-tdTomato fluorescent reporter in NANOS3 locus gave rise to cloned porcine foetuses by nuclear transfer. In summary, for major livestock mammals pluripotent stem cells related to the formative embryonic disc are reliably established using a common and defined signaling environment.

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Center for Stem Cell Biology and Regenerative Medicine

Division of Stem Cell Aging Medicine
幹細胞加齢医学分野

Professor Emi K. Nishimura, M.D., Ph.D.

教 授 博士(医学) 西 村 栄 美

Stem cell systems play fundamental roles in sustaining tissue turnover and homeostasis. Our goal is to understand the mechanisms of tissue aging and cancer development in mammals and to apply that knowledge to develop strategies to resist against tissue/organ aging, cancer development and other relevant diseases associated with aging. We further aim to apply this knowledge to drug discovery and the prevention and treatment of age-associated diseases.

1. Distinct types of stem cell divisions determine organ regeneration and aging in hair follicles

Matsumura H, Liu N[1], Nanba D, Ichinose S[2], Takada A[1], Kurata S[3], Morinaga H, Mohri Y, De Arcangelis A[4], Ohno S[5], and Nishimura EK.; [1]Department of Stem Cell Biology, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan; [2]Research Center for Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan; [3]Beppu Garden-Hill Clinic, Kurata Clinic, Beppu City, Japan; [4]Institut de Génétique et de Biologie Moléculaire et Cellulaire, Department of Development and Stem Cells, CNRS UMR7104, Inserm U1258, Université de Strasbourg, Illkirch, France; [5]Department of Molecular Biology, Yokohama City University School of Medicine, Kanazawa, Yokohama, Japan.

Hair follicles, mammalian mini-organs that grow hair, miniaturize during aging, leading to hair thinning and loss. Here we report that hair follicle stem cells (HFSCs) lose their regenerative capabilities during aging owing to the adoption of an atypical cell division program. Cell fate tracing and cell division axis analyses revealed that while HFSCs in young mice undergo typical symmetric and asymmetric cell divisions to regenerate hair follicles, upon aging or stress, they adopt an atypical 'stress-responsive type of asymmetric cell division. This type of division is accompanied by the destabilization of hemidesmosomal protein COL17A1 and cell polarity protein aPKCλ and generates terminally differentiating epidermal cells instead of regenerating the hair follicle niche. With the repetition of these atypical divisions, HFSCs detach from the basal membrane causing their exhaustion, elimination and organ aging. The experimentally induced stabilization of COL17A1 rescued organ homeostasis through aPKCλ stabilization. These results demonstrate that distinct stem cell division programs may govern tissue and organ aging.

2. Obesity accelerates hair thinning by stem cell-centric converging mechanisms

Morinaga H, Mohri Y, Grachtchouk M[1], Asakawa K, Matsumura H, Oshima M[2], Takayama N[3], Kato T, Nishimori Y, Sorimachi Y[4], Takubo K[4], Suganami T[5], Iwama A[2], Iwakura Y[6], Dlugosz A.A[1], and Nishimura E.K.; [1]Department of Dermatology, University of Michigan Medical School, Ann Arbor, MI, USA; [2]Division of Stem Cell and Molecular Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan; [3]Department of Regenerative Medicine, Graduate School of Medicine, Chiba University, Chiba, Japan; [4]Department of Stem Cell Biology, Research Institute National

Center for Global Health and Medicine, Tokyo, Japan; [5]Department of Molecular Medicine and Metabolism, Research Institute of Environmental Medicine, Nagoya University, Nagoya, Japan; [6]Centre for Animal Disease Models, Research Institute for Biomedical Sciences, Tokyo University of Science, Chiba, Japan.

Obesity is a worldwide epidemic that predisposes individuals to many age-associated diseases, but its exact effects on organ dysfunction are largely unknown. Hair follicles—mini-epithelial organs that grow hair—are miniaturized by ageing to cause hair loss through the depletion of hair follicle stem cells (HFSCs). Here we report that obesity-induced stress, such as that induced by a high-fat diet (HFD), targets HFSCs to accelerate hair thinning. Chronological gene expression analysis revealed that HFD feeding for four consecutive days in young mice directed activated HFSCs towards epidermal keratinization by generating excess reactive oxygen species, but did not reduce the pool of HFSCs. Integrative analysis using stem cell fate tracing, epigenetics and reverse genetics showed that further feeding with an HFD subsequently induced lipid droplets and NF-κB activation within HFSCs via autocrine and/or paracrine IL-1R signalling. These integrated factors converge on the marked inhibition of Sonic hedgehog (SHH) signal transduction in HFSCs, thereby further depleting lipid-laden HFSCs through their aberrant differentiation and inducing hair follicle miniaturization and eventual hair loss. Conversely, transgenic or pharmacological activation of SHH rescued HFD-induced hair loss. These data collectively demonstrate that stem cell inflammatory signals induced by obesity robustly represses organ regeneration signals to accelerate the miniaturization of mini-organs, and suggests the importance of daily prevention of organ dysfunction.

3. Dynamic stem cell selection safeguards the genomic integrity of the epidermis

Kato T, Liu N, Morinaga H, Asakawa K, Muraguchi T, Muroyama Y, Shimokawa M, Matsumura H, Nishimori Y, Tan LJ, Hayano M[1,2,3], Sinclair DA[1,4], Mohri Y, Nishimura EK. ; [1]Department of Genetics, Blavatnik Institute, Paul F. Glenn Center for Biology of Aging Research, Harvard Medical School, Boston, MA, USA; [2]Department of Neuropsychiatry, Keio University School of Medicine, Shinjuku-ku, Tokyo, Japan; [3]Faculty of Science and Technology, Keio University, Yokohama, Japan; [4]Laboratory for Ageing Research, Department of Pharmacology, School of Medical Sciences, The University of New South Wales, Sydney, New South Wales, Australia

Maintaining genomic integrity and stability is crucial for life; yet, no tissue-driven mechanism that robustly safeguards the epithelial genome has been discovered. Epidermal stem cells (EpiSCs) continuously replenish the stratified layers of keratinocytes that protect organisms against various environmental stresses. To study the dynamics of DNA-damaged cells in tissues,we devised an in vivo fate tracing system for EpiSCs with DNA double-strand breaks (DSBs) and demonstrated that those cells exit from their niches. The clearance of EpiSCs with DSBs is caused by selective differentiation and delamination through the DNA damage response (DDR)-p53-Notch/p21 axis, with the downregulation of ITGB1. Moreover, concomitant enhancement of symmetric cell divisions of surrounding stem cells indicates that the selective elimination of cells with DSBs is coupled with the augmented clonal expansion of intact stem cells. These data collectively demonstrate that tissue autonomy through the dynamic coupling of cell-autonomous and non-cell-autonomous mechanisms coordinately maintains the genomic quality of the epidermis.

4. Stem cell spreading dynamics intrinsically differentiate acral melanomas from nevi.

Eshiba S, Namiki T[1], Mohri Y, Aida T[2,3], Serizawa N[4], Shibata T[5], Morinaga H, Nanba D, Hiraoka Y[2,3], Tanaka K[2], Miura K[6], Tanaka M[7], Uhara H[8], Yokozeki H[1], Saida T[9], Nishimura EK; [1]Department of Dermatology, Tokyo Medical and Dental University Graduate School and Faculty of Medicine,Tokyo, Japan; [2]Department of Molecular Neuroscience, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan; [3]Laboratory of Genome Editing for Biomedical Research, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan; [4]Department of Stem Cell Biology, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan; [5]Medical Corporation Shibata Dermatology Clinic, Osaka, Japan; [6]Department of Pathology, Tokyo Medical and Dental University Graduate School and Faculty of Medicine, Tokyo, Japan; [7]Department of Dermatology, Tokyo Women's Medical University Medical Center East, Tokyo, Japan; [8]Department of Dermatology, Sapporo Medical University School of Medicine, Hokkaido, Japan; [9]Shinshu University, Saitama, Japan

Early differential diagnosis between malignant and benign tumors and their underlying intrinsic differences are the most critical issues for life-threatening cancers. To study whether human acral melanomas, deadly cancers that occur on non-hair-bearing skin, have distinct origins that underlie their invasive capability, we develop fate-tracing technologies of melanocyte stem cells in sweat glands (glandular McSCs) and in melanoma models in mice and com-

pare the cellular dynamics with human melanoma. Herein, we report that glan-dular McSCs self-renew to expand their migratory progeny in response to genotoxic stress and trauma to generate invasive melanomas in mice that mimic human acral melanomas. The analysis of melanocytic lesions in human volar skin reveals that genetically unstable McSCs expand in sweat glands and in the surrounding epidermis in melanomas but not in nevi. The detection of such cell spreading dynamics provides an innovative method for an early differential diagnosis of acral melanomas from nevi.

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Center for Stem Cell Biology and Regenerative Medicine

Division of Somatic Stem Cell Research
体性幹細胞研究分野

Associate Professor Tokiko Nagamura-Inoue, M.D., Ph.D. | 准教授 博士（医学） 長 村 登紀子

Somatic stem cells, which are derived from mesoderm, include mesenchymal stromal cells (MSCs), blood cells, and other mesenchymal tissues. MSCs exist in the interstitium of systemic organs; they have self-renewal ability, migrate to the sites of inflammation and tissue damage, and exert anti-inflammatory effects and tissue-repair ability. Among various somatic stem cells, we focused on umbilical cord blood (CB) and umbilical cord-derived MSCs (UC-MSCs). Collaborating with IMSUT-CORD (CB and UC bank), we explored new immune and regenerative gene/cell therapies using CB and UC tissue with high standards of quality and safety. Regarding the quality and safety standards, we have begun to use the new IMSUT-HLC cell processing facility and aim to obtain a manufacturing license.

Cord blood and umbilical cord–derived cells for immune-cell therapy and regenerative medicine

Takahashi A, Hori A, Miharu Y, Yamamoto Y, Nagaya N, Ogami K, Okamura K, Nagamura-Inoue T

We focused on umbilical cord blood (CB) and umbilical cord-derived MSCs (UC-MSCs) of perinatal tissues, among other somatic stem cells. By collaborating with IMSUT-CORD (CB and UC bank), we explored new immune and regenerative gene/cell therapies using CB and UC with high quality and safety standards. For the high quality and safety standards we began to use a new IMSUT-HLC cell processing facility, for which we aim to obtain a manufacturing license. In addition, it is our mission to keep the IMSUT-HLC cell processing facility clean and functional to enable high-quality manufacturing for gene and cell therapy.

In the basic research, our interest is to elucidate the mechanism of immune modulation via monocyte/microglia polarization and the migration and chemotaxis ability of UC-MSCs compared against those of bone marrow and adipose-derived MSCs.

Publications

1) Kurogi H, Takijiri T, Sakumoto M, Isogai M, Takahashi A, Okubo T, Koike T, Yamada T, Nagamura-Inoue T, Yumoto M., Study on the umbilical cord mesenchymal stem cell-manufacturing using clinical grade culture medium Tissue Eng Part C Methods. Online ahead of print. 2022 Jan 12.
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3) Mukai T, Di Martino E, Tsuji S, Blomgren K, Nagamura-Inoue T, Ådén U. Umbilical cord-derived mesenchymal stromal cells immunomodulate and restore actin dynamics and phagocytosis of LPS-activated microglia via PI3K/Akt/Rho GTPase pathway. Cell Death Discov. 7:46, 2021

Center for Stem Cell Biology and Regenerative Medicine

FACS Core Laboratory
FACS コアラボラトリー

Professor　Atsushi Iwama, M.D., Ph.D.

教　授　博士(医学)　　岩　間　厚　志

The FACS Core Laboratory provides high quality, cost-effective and state-of-the-art flow cytometry (FCM) services for internal and external researchers. We offer assistance in the following areas: (1) initial project planning (2) antibody panel design and optimization (3) instrument operation and maintenance (4) data analysis.

Instruments at the FACS Core Laboratory

For cell sorting, the FACS Core is equipped with Three BD FACS Aria Cell sorters from BD Biosciences and one SH800 cell sorter from SONY. For cell analysis, the FACS Core Laboratory is equipped with three benchtop analyzers.

FCM usage performance in 2021

FCM analysis and sorting is performed either by the FACS Core staff or by trained users. There were about 3,700 cases of FCM use in 2021.

Seminar and Training

The FACS Core provided training and technical seminars about the theory and practical use of the FCM technology to students, fellows, and principal investigators at IMSUT.

International Research Center for Infectious Diseases

Department of Special Pathogens
高病原性感染症系

Associate Professor	Takeshi Ichinohe, Ph.D.		准教授	博士(工学)	一戸 猛志
Visiting Professor	Masaki Imai, D.V.M., Ph.D.		客員教授	博士(獣医学)	今井 正樹
Visiting Professor	Seiya Yamayoshi, D.V.M., Ph.D.		客員教授	博士(医学)	山吉 誠也

Highly pathogenic viral agents causing emerging infectious diseases are of concern not only to public health but also as possible biological weapons. The ultimate goal of our research is to unlock the secrets of the pathogenicity of such viruses in humans and to develop effective vaccines and antiviral compounds against these pathogens. We have been investigating the molecular basis of the replication cycle and extreme virulence of special pathogens, using Ebolavirus, influenza virus, and SARS-CoV-2 as model viral agents.

Characterization of a new SARS-CoV-2 variant that emerged in Brazil.

Masaki Imai, Peter J. Halfmann[1], Seiya Yamayoshi, Kiyoko Iwatsuki-Horimoto, Shiho Chiba[1], Tokiko Watanabe[2], Noriko Nakajima[3], Mutsumi Ito, Makoto Kurod[1], Maki Kiso, Tadashi Maemura[1], Kenta Takahashi[3], Samantha Loeber[4], Masato Hatta[1], Michiko Koga[5,6], Hiroyuki Nagai[6], Shinya Yamamoto[5,6], Makoto Saito[5,6], Eisuke Adachi[6], Osamu Akasaka[7], Morio Nakamura[8], Ichiro Nakachi[9], Takayuki Ogura[10], Rie Baba[9], Kensuke Fujita[10], Junichi Ochi[11], Keiko Mitamura[12], Hideaki Kato[13,14], Hideaki Nakajima[14], Kazuma Yagi[15], Shin-ichiro Hattori[16], Kenji Maeda[16], Tetsuya Suzuki[17], Yusuke Miyazato[17], Riccardo Valdez[18], Carmen Gherasim[18], Yuri Furusawa, Moe Okuda, Michiko Ujie, Tiago J. S. Lopes, Atsuhiro Yasuhara, Hiroshi Ueki, Yuko Sakai-Tagawa, Amie J. Eisfeld[1], John J. Baczenas[19,20], David A. Baker[19], Shelby L. O'Connor[19,20], David H. O'Connor[19,20], Shuetsu Fukushi[21], Tsuguto Fujimoto[22], Yudai Kuroda[23], Aubree Gordon[24], Ken Maeda[23], Norio Ohmagari[17], Norio Sugaya[25], Hiroshi Yotsuyanagi[5,6], Hiroaki Mitsuya[16,26], Tadaki Suzuki[3], and Yoshihiro Kawaoka[1,27]

[1]Influenza Research Institute, Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin−Madison
[2]Department of Molecular Virology, Research Institute for Microbial Diseases, Osaka University
[3]Department of Pathology, National Institute of Infectious Diseases
[4]Department of Surgical Sciences, School of Veterinary Medicine, University of Wisconsin−Madison
[5]Division of Infectious Diseases, Advanced Clinical Research Center, Institute of Medical Science, University of Tokyo
[6]Department of Infectious Diseases and Applied Immunology, IMSUT Hospital of The Institute of Medical Science, University of Tokyo
[7]Emergency Medical Center, Fujisawa City Hospital
[8]Department of Pulmonary Medicine, Tokyo Saiseikai Central Hospital
[9]Pulmonary Division, Department of Internal Medicine, Saiseikai Utsunomiya Hospital
[10]Department of Emergency & Intensive Care, Saiseikai Utsunomiya Hospita
[11]Department of Respiratory Medicine, Eiju General Hospital
[12]Division of Infection Control, Eiju General Hospital
[13]Infection Prevention and Control Department, Yokohama City University Hospital
[14]Department of Hematology and Clinical Immunol-

ogy, Yokohama City University School of Medicine
[15]Department of Pulmonary Medicine, Keiyu Hospital
[16]Department of Refractory Viral Infections, National Center for Global Health and Medicine Research Institute
[17]Disease Control and Prevention Center, National Center for Global Health and Medicine Hospital
[18]Department of Pathology, University of Michigan
[19]Department of Pathology and Laboratory Medicine, University of Wisconsin−Madison
[20]Wisconsin National Primate Research Center, University of Wisconsin−Madison
[21]Department of Virology 1, National Institute of Infectious Diseases
[22]Center for Emergency Preparedness and Response, National Institute of Infectious Diseases
[23]Department of Veterinary Science, National Institute of Infectious Diseases
[24]Department of Epidemiology, School of Public Health, University of Michigan
[25]Department of Pediatrics, Keiyu Hospital
[26]Experimental Retrovirology Section, HIV and AIDS Malignancy Branch, National Cancer Institute, NIH
[27]Department of Special Pathogens, International Research Center for Infectious Diseases, Institute of Medical Science, University of Tokyo

The spike (S) protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) plays a key role in viral infectivity. It is also the major antigen stimulating the host's protective immune response, specifically, the production of neutralizing antibodies. Recently, a new variant of SARS-CoV-2 possessing multiple mutations in the S protein, designated P.1, emerged in Brazil. Here, we characterized a P.1 variant isolated in Japan by using Syrian hamsters, a well-established small animal model for the study of SARS-CoV-2 disease (COVID-19). In hamsters, the variant showed replicative abilities and pathogenicity similar to those of early and contemporary strains (i.e., SARS-CoV-2 bearing aspartic acid [D] or glycine [G] at position 614 of the S protein). Sera and/or plasma from convalescent patients and BNT162b2 messenger RNA vaccinees showed comparable neutralization titers across the P.1 variant, S-614D, and S-614G strains. In contrast, the S-614D and S-614G strains were less well recognized than the P.1 variant by serum from a P.1-infected patient. Prior infection with S-614D or S-614G strains efficiently prevented the replication of the P.1 variant in the lower respiratory tract of hamsters upon reinfection. In addition, passive transfer of neutralizing antibodies to hamsters infected with the P.1 variant or the S-614G strain led to reduced virus replication in the lower respiratory tract. However, the effect was less pronounced against the P.1 variant than the S-614G strain. These findings suggest that the P.1 variant may be somewhat antigenically different from the early and contemporary strains of SARS-CoV-2.

Antibody titers against SARS-CoV-2 decline, but do not disappear for several months.

Seiya Yamayoshi, Atsuhiro Yasuhara, Mutsumi Ito, Osamu Akasaka, Morio Nakamura, Ichiro Nakachi, Michiko Koga, Keiko Mitamura, Kazuma Yagi, Kenji Maeda, Hideaki Kato, Masanori Nojima[1], David Pattinson[2], Takayuki Ogura, Rie Baba, Kensuke Fujita, Hiroyuki Nagai, Shinya Yamamoto, Makoto Saito, Eisuke Adachi, Junichi Ochi, Shin-ichiro Hattori, Tetsuya Suzuki, Yusuke Miyazato, Shiho Chiba, Moe Okuda, Jurika Murakami, Taiki Hamabata, Kiyoko Iwatsuki-Horimoto, Hideaki Nakajima, Hiroaki Mitsuya, Norio Omagari, Norio Sugaya, Hiroshi Yotsuyanagi, Yoshihiro Kawaoka

To develop an effective vaccine against a novel viral pathogen, it is important to understand the longitudinal antibody responses against its first infection. Here we performed a longitudinal study of antibody responses against SARS-CoV-2 in symptomatic patients. Sequential blood samples were collected from 39 individuals at various timepoints between 0 and 154 days after onset. IgG or IgM titers to the receptor binding domain (RBD) of the S protein, the ectodomain of the S protein, and the N protein were determined by using an ELISA. Neutralizing antibody titers were measured by using a plaque reduction assay. The IgG titers to the RBD of the S protein, the ectodomain of the S protein, and the N protein peaked at about 20 days after onset, gradually decreased thereafter, and were maintained for several months after onset. Extrapolation modeling analysis suggested that the IgG antibodies were maintained for this amount of time because the rate of reduction slowed after 30 days post-onset. IgM titers to the RBD decreased rapidly and disappeared in some individuals after 90 days post-onset. All patients, except one, possessed neutralizing antibodies against authentic SARS-CoV-2, which they retained at 90 days after onset. The highest antibody titers in patients with severe infections were higher than those in patients with mild or moderate infections, but the decrease in antibody titer in the severe infection cohort was more remarkable than that in the mild or moderate infection cohort.

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International Research Center for Infectious Diseases

Department of Infectious Disease Control
感染制御系

Professor	Yasushi Kawaguchi, D.V.M., Ph.D.	教　授	博士(獣医学)	川　口　　寧
Associate Professor	Akihisa Kato, Ph.D.	准教授	博士(医学)	加　藤　哲　久
Assistant Professor	Naoto Koyanagi, Ph.D.	助　教	博士(生命科学)	小　柳　直　人
Assistant Professor	Yuhei Maruzuru, Ph.D.	助　教	博士(生命科学)	丸　鶴　雄　平

Our special interest is focused upon searching for effective methods to protect or control viral infection by using accumulated knowledge based on molecular pathogenicity, and developing novel anti-viral drugs and attenuated strains for novel vaccines. The works have been conducted by close collaboration with Division of Molecular Virology, Department of Microbiology and Immunology.

1. Prohibitin-1 contributes to the cell-to-cell transmission of herpes simplex virus 1 via the MAPK/ERK signaling pathway

Mizuki Watanabe, Jun Arii[1], Kosuke Takeshima, Ayano Fukui, Masayuki Shimojima[2], Hiroko Kozuka-Hata[3], Masaaki Oyama[3], Takeharu Minamitani[4], Teruhito Yasui[4], Yuji Kubota[5], Mutsuhiro Takekawa[5], Isao Kosugi[6], Yuhei Maruzuru, Naoto Koyanagi, Akihisa Kato, Yasuko Mori[1], and Yasushi Kawaguchi: [1]Division of Clinical Virology, Center for Infectious Diseases, Kobe University Graduate School of Medicine, Kobe, Hyogo [2]Department of Virology I, National Institute of Infectious Diseases, Tokyo [3]Medical Proteomics Laboratory, The Institute of Medical Science, The University of Tokyo [4]Laboratory of Infectious Diseases and Immunity, National Institutes of Biomedical Innovation, Health, and Nutrition, Ibaraki, Osaka [5]Division of Cell Signaling and Molecular Medicine, Department of Basic Medical Sciences, Institute of Medical Science, The University of Tokyo [6]Department of Regenerative and Infectious Pathology, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka

Viral cell-to-cell spread, a method employed by several viral families for entrance via cell junctions, is highly relevant to the pathogenesis of various viral infections. Cell-to-cell spread of herpes simplex virus 1 (HSV-1) is known to depend greatly on envelope glycoprotein E (gE). However, the molecular mechanism by which gE acts in HSV-1 cell-to-cell spread and the mechanisms of cell-to-cell spread by other herpesviruses remain poorly understood. Here, we describe our identification of prohibitin-1 as a novel gE-interacting host cell protein. Ectopic expression of prohibitin-1 increased gE-dependent HSV-1 cell-to-cell spread. As observed with the gE-null mutation, decreased expression or pharmacological inhibition of prohibitin-1 reduced HSV-1 cell-to-cell spread without affecting the yield of virus progeny. Similar effects were produced by pharmacological inhibition of the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway, wherein prohibitin-1 acts as a protein scaffold and is required for induction of this pathway. Furthermore, artificial activation of the MAPK/ERK pathway restored HSV-1 cell-to-cell spread impaired by the gE-null mutation. Notably, pharmacological inhibition of prohibitins or the MAPK/ERK pathway reduced viral cell-to-cell spread of representative members in all

herpesvirus subfamilies. Our results suggest that prohibitin-1 contributes to gE-dependent HSV-1 cell-to-cell spread via the MAPK/ERK pathway and that this mechanism is conserved throughout the Herpesviridae, whereas gE is conserved only in the Alphaherpesvirinae subfamily.

IMPORTANCE Herpesviruses are ubiquitous pathogens of various animals, including humans. These viruses primarily pass through cell junctions to spread to uninfected cells. This method of cell-to-cell spread is an important pathogenic characteristic of these viruses. Here, we show that the host cell protein prohibitin-1 contributes to HSV-1 cell-to-cell spread via a downstream intracellular signaling cascade, the MAPK/ERK pathway. We also demonstrate that the role of the prohibitin-1-mediated MAPK/ERK pathway in viral cell-to-cell spread is conserved in representative members of every herpesvirus subfamily. This study has revealed a common molecular mechanism of the cell-to-cell spread of herpesviruses.

2. Role of the DNA binding activity of herpes simplex virus 1 VP22 in evading AIM2-dependent inflammasome activation induced by the virus

Yuhei Maruzuru, Naoto Koyanagi, Akihisa Kato, and Yasushi Kawaguchi

AIM2 is a cytosolic DNA sensor of the inflammasome, which induces critical innate immune responses against various invading pathogens. Earlier biochemical studies showed that the binding of AIM2 to DNA triggered the self-oligomerization of AIM2, which is essential for AIM2 inflammasome activation. We recently reported that VP22, a virion tegument protein of herpes simplex virus 1 (HSV-1), inhibited activation of the AIM2 inflammasome in HSV-1-infected cells by preventing AIM2 oligomerization. VP22 binds non-specifically to DNA; however, its role in HSV-1 replication is unclear. We investigated the role of VP22 DNA binding activity in the VP22-mediated inhibition of AIM2 inflammasome activation. We identified a VP22 domain encoded by amino acids 227 to 258 as the minimal domain required for its binding to DNA in vitro Consecutive alanine substitutions in this domain substantially impaired the DNA binding activity of VP22 in vitro and attenuated the inhibitory effect of VP22 on AIM2 inflammasome activation in an AIM2 inflammasome reconstitution system. The inhibitory effect of VP22 on AIM2 inflammasome activation was completely abolished in macrophages infected with a recombinant virus harboring VP22 with one of the consecutive alanine substitutions, similar to the effect of a VP22-null mutant virus. These results suggested that the DNA binding activity of VP22 is critical for VP22-mediated AIM2 inflammasome activation in HSV1-infected cells.

IMPORTANCE VP22, a major component of the HSV-1 virion tegument, is conserved in alphaherpesviruses and has structural similarity to ORF52, a component of the virion tegument that is well-conserved in gammaherpesviruses. Although the potential DNA binding activity of VP22 was discovered decades ago, its significance in the HSV-1 life cycle is poorly understood. Here, we show that the DNA binding activity of VP22 is critical for the inhibition of AIM2 inflammasome activation induced in HSV-1-infected cells. This is the first report to show a role for the DNA binding activity of VP22 in the HSV-1 life cycle, allowing the virus to evade AIM2 inflammasome activation, which is critical for its replication in vivo.

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International Research Center for Infectious Diseases

Department of Infectious Disease Control Division of Viral Infection

感染制御系・ウイルス学分野

Associate Professor　Takeshi Ichinohe, Ph.D.

准教授　博士（工学）　一　戸　猛　志

We focus on understanding how viruses are recognized by NLRP3 inflammasome and how the innate recognition receptor controls antigen-specific adaptive immune responses. We study immune responses to influenza viruses in the lung. Our recent focus also includes the study of how microbiota regulates adaptive immune responses to these pathogens. Our ultimate goal is to utilize the knowledge we gain through these areas of research in the rational design of effective vaccines for the prevention of infectious diseases.

1. Oral Bacteria Combined with an Intranasal Vaccine Protect from Influenza A Virus and SARS-CoV-2 Infection.

Nagai M, Moriyama M, and Ichinohe T.

The gut microbiota plays a critical role in the induction of adaptive immune responses to influenza virus infection. However, the role of nasal bacteria in the induction of the virus-specific adaptive immunity is less clear. Here, we found that disruption of nasal bacteria by intranasal application of antibiotics before influenza virus infection enhanced the virus-specific antibody response in a MyD88-dependent manner. Similarly, disruption of nasal bacteria by lysozyme enhanced antibody responses to intranasally administered influenza virus hemagglutinin (HA) vaccine in a MyD88-dependent manner, suggesting that intranasal application of antibiotics or lysozyme could release bacterial pathogen-associated molecular patterns (PAMPs) from disrupted nasal bacteria that act as mucosal adjuvants by activating the MyD88 signaling pathway. Since commensal bacteria in the nasal mucosal surface were significantly lower than those in the oral cavity, intranasal administration of HA vaccine alone was insufficient to induce the vaccine-specific antibody response. However, intranasal supplementation of cultured oral bacteria from a healthy human volunteer enhanced antibody responses to an intranasally administered HA vaccine. Finally, we demonstrated that oral bacteria combined with an intranasal vaccine protect from influenza virus and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Our results reveal the role of nasal bacteria in the induction of the virus-specific adaptive immunity and provide clues for developing better intranasal vaccines.

Publications

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International Research Center for Infectious Diseases

Department of Infectious Disease Control Division of Systems Virology
感染制御系・システムウイルス学分野

Associate Professor	Kei Sato, Ph.D.	准教授 博士(医学)	佐 藤 佳
Project Assistant Professor	Jumpei Ito, Ph.D., D.V.M.	特任助教 博士(理学)	伊 東 潤 平

The aim of our laboratory is to expand the knowledge and methodology on virology, which were unable to shed light on by conventional experimental approach. To investigate the co-evolutionary relationship between viruses and hosts, we perform bioinformatic and molecular phylogenetic analyses as well as experimental virology. The interdisciplinary investigations based on experimental virology and other scientific fields/methods will pioneer a new science for deeply understanding infectious diseases.

1. Co-evolution of retroviruses and mammals

Jumpei Ito, Yoriyuki Konno, Shumpei Nagaoka, Izumi Kimura, Hirofumi Aso, Keiya Uriu, Yusuke Kosugi, Narumi Suzuki, Yuka Masuda, Kei Sato

As the hosts of lentiviruses, almost 40 species of felids (family Felidae) are distributed around the world, and more than 20 feline species test positive for feline immunodeficiency virus (FIV), a lineage of lentiviruses. These observations suggest that FIVs globally infected a variety of feline species through multiple cross-species transmission events during a million-year history. Cellular restriction factors potentially inhibit lentiviral replication and limit cross-species lentiviral transmission, and cellular APOBEC3 deaminases are known as a potent restriction factor. In contrast, lentiviruses have evolutionary- acquired viral infectivity factor (Vif) to neutralize the APOBEC3-mediated antiviral effect. Because the APOBEC3-Vif interaction is strictly specific for viruses and their hosts, a com- prehensive investigation focusing on Vif-APOBEC3 interplay can provide clues that will elucidate the roles of this virus-host interplay on cross-species transmission of lentiviruses. Here, we performed a comprehensive investigation with 144 patterns of a round robin test using 18 feline APOBEC3Z3 genes, an antiviral APOBEC3 gene in felid, and 8 FIV Vifs and derived a matrix showing the interplay between feline APOBEC3Z3 and FIV Vif. We particularly focused on the interplay between the APOBEC3Z3 of three felids (domestic cat, ocelot, and Asian golden cat) and an FIV Vif (strain Petaluma), and revealed that residues 65 and 66 of the APOBEC3Z3 protein of multiple felids are responsible for the counteraction triggered by FIV Petaluma Vif. Altogether, our findings can be a clue to elucidate not only the scenarios of the cross-species transmissions of FIVs in felids but also the evolutionary interaction between mammals and lentiviruses.

2. Evolution of SARS-CoV-2

Jumpei Ito, Daichi Yamasoba, Izumi Kimura, Keiya Uriu, Yusuke Kosugi, Kei Sato.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a causative agent of coronavirus disease 2019 (COVID-19). SARS-CoV-2 emerged at the end of 2019 and has spread all over the world. In the last two years, more than 300 million people are infected with this virus and more than 5 million people

died of COVID-19, meaning that COVID-19 is ongoing pandemic and a most urgent and crucial problem in the current human society. To proceed and accelerate COVID-19-releated researches in Japan, we launched a consortium, called "The Genotype to Phenotype Japan (G2P-Japan) Consortium" in January 2021. As of January 2022, more than 10 principal investigators in Japan join this consortium and proceed fruitful collaboration. We aim to elucidate the virological characteristics of the SARS-CoV-2 variants continuously emerging in the world.

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International Research and Development Center for Mucosal Vaccines

Division of Mucosal Barriology
粘膜バリア学分野

Professor	Cevayir Coban, M.D., Ph.D. (Clinical Microbiology)	教　授	博士(医学)(臨床微生物学位)　チョバン　ジェヴァイア
Visiting Professor	Koji Hase, Ph.D.	客員教授	博士(薬学)　長　谷　耕　二

The goal of this Division is to understand the interaction between the mucosal tissues and the immune barrier system during homeostasis as well as pathological conditions that occur during microbial infection and/or other immunological disorders. The final aim is to develop novel mucosal vaccines.

1. Symbiotic polyamine metabolism regulates epithelial proliferation and macrophage differentiation in the colon.

Atsuo Nakamura[1,2], Shin Kurihara[3,4], Daisuke Takahashi[1], Wakana Ohashi[1], Yutaka Nakamura[1], Shunsuke Kimura[1,5], Masayoshi Onuki[1], Aiko Kume[2], Yukiko Sasazawa[6], Yukihiro Furusawa[1,7], Yuuki Obata[1,8], Shinji Fukuda[5,9,10,11], Shinji Saiki[6], Mitsuharu Matsumoto[12], Koji Hase[13,14]: [1]Division of Biochemistry, Faculty of Pharmacy and Graduate School of Pharmaceutical Science, Keio University, Minato-ku, Tokyo, Japan, [2]Dairy Science and Technology Institute, Kyodo Milk Industry Co., Ltd., Hinode-machi, Nishitama-gun, Tokyo, Japan, [3]Faculty of Bioresources and Environmental Sciences, Ishikawa Prefectural University, Nonoichi, Ishikawa, Japan, [4]Faculty of Biology-Oriented Science and Technology, Kindai University, Kinokawa, Wakayama, Japan, [5]PRESTO, Japan Science and Technology Agency, Kawaguchi, Saitama, Japan, [6]Department of Neurology, Graduate School of Medicine, Juntendo University, Bunkyo-ku, Tokyo, Japan, [7]Department of Liberal Arts and Sciences, Toyama Prefectural University, Kurokawa, Toyama, Japan, [8]The Francis Crick Institute, London, UK, [9]Institute for Advanced Biosciences, Keio University, Tsuruoka, Yamagata, Japan, [10]Transborder Medical Research Center, University of Tsukuba, Tsukuba, Ibaraki, Japan, [11]Intestinal Microbiota Project, Kanagawa Institute of Industrial Science and Technology, Kawasaki, Kanagawa, Japan, [12]Dairy Science and Technology Institute, Kyodo Milk Industry Co., Ltd., Hinode-machi, Nishitama-gun, Tokyo, Japan, [13]Division of Biochemistry, Faculty of Pharmacy and Graduate School of Pharmaceutical Science, Keio University, Minato-ku, Tokyo, Japan, [14]International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo (IMSUT), Bunkyo-ku, Tokyo, Japan.

Intestinal microbiota-derived metabolites have biological importance for the host. Polyamines, such as putrescine and spermidine, are produced by the intestinal microbiota and regulate multiple biological processes. Increased colonic luminal polyamines promote longevity in mice. However, no direct evidence has shown that microbial polyamines are incorporated into host cells to regulate cellular responses. Here, we show that microbial polyamines reinforce colonic epithelial proliferation and regulate macrophage differentiation. Colonisation by wild-type, but not polyamine biosynthesis-deficient, Escherichia coli in

germ-free mice raises intracellular polyamine levels in colonocytes, accelerating epithelial renewal. Commensal bacterium-derived putrescine increases the abundance of anti-inflammatory macrophages in the colon. The bacterial polyamines ameliorate symptoms of dextran sulfate sodium-induced colitis in mice. These effects mainly result from enhanced hypusination of eukaryotic initiation translation factor. We conclude that bacterial putrescine functions as a substrate for symbiotic metabolism and is further absorbed and metabolised by the host, thus helping maintain mucosal homoeostasis in the intestine.

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International Research and Development Center for Mucosal Vaccines

Division of Innate Immune Regulation
自然免疫制御分野

Project Professor	Satoshi Uematsu, M.D., Ph.D.	特任教授　博士(医学)	植　松　　智
Project Assistant Professor	Kosuke Fujimoto, M.D., Ph.D.	特任助教　博士(医学)	藤　本　康　介

Innate immunity is the 'gateway' of immune response. By controlling innate immunity, it is thought that the whole immunity is controllable. Our major focus is the elucidation and understanding of molecular function of the innate immune cells in small intestine for the development of mucosal vaccine against infectious diseases and mucosal immune therapy for inflammatory bowel diseases. We also analyze intestinal microbiome by developing new informatics method. We will develop new therapeutic strategies against various dysbiosis-related diseases targeting on intestinal microbiota by using our mucosal vaccine.

1. Development of next-generation mucosal vaccine against infectious diseases

Kosuke Fujimoto[1], Satoshi Uematsu[1]
[1]Division of Innate Immune regulation, International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo.

A next-generation vaccine strategy capable of inducing both systemic and mucosal immunity is awaited. We showed that intramuscular vaccination with a combination of CpG oligodeoxynucleotides and curdlan as adjuvants systemically induced antigen-specific IgA and IgG production in mice. After priming, markedly high titers and long-lasting antigen-specific IgA and helper T-cell responses including Th1 and Th17 responses in the mucosa were acquired by antigen boosting of the target organs. This immunization effectively regulated *Streptococcus pneumoniae* infection in mice. The patent of this new vaccine strategy was granted in 2019 in Japan, in 2020 in US and in 2021 in Europe. We are currently conducting monkey experiments for formulation in human on the basis of collaboration with Mitsubishi Tanabe Pharmaceutical company by using PspA, a universal Ag of *S. pneumoniae*. We are also collaborating with Medicago in Canada and are developing an IgA-inducing mucosal vaccine with this system by using SARS-CoV-2 Viral-like particle as an antigen which have been developed by Medicago.

2. Development of next-generation mucosal vaccine against intestinal pathobiont

Kosuke Fujimoto[1], Satoshi Uematsu[1]
[1]Division of Innate Immune regulation, International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo

Dysbiosis is associated with many diseases, including obesity and diabetes. Therefore, controlling dysbiosis and the bacteria responsible for it are key factors in successfully treating commensal microbiome-mediated diseases. Several lines of evidence suggest that increased levels of *Clostridium ramosum*, a representative intestinal microbiome, are associated with human obesity. *C. ramosum* is associated with clinical signs of metabolic disorders using a gnotobiotic mouse colonized with *C. ramosum* alone and a simplified human intestinal microbiome containing *C. ramosum*. Futhermore, it has been shown that *C. ramosum* numbers were higher in mice fed a HFD

compared with those fed a normal-fat diet (NFD), and that this contributed to increased expression of solute carrier family 2, member 2 (*Slc2a2*) (otherwise called *Glut2)* in the small intestinal mucosa. We have shown that vaccination for *C. ramosum* with our method alleviated high-fat diet-induced obesity in mice by controlling the number of *C. ramosum* in the mucosa. We are searching for the best vaccine antigen for *C. ramosum* in order to develop an obesity vaccine.

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International Research and Development Center for Mucosal Vaccines

Division of Clinical Vaccinology
臨床ワクチン学分野

Project Professor	Kohtaro Fujihashi, D.D.S., Ph.D.		特任教授	博士（歯学）	藤　橋　浩太郎
Project Associate Professor	Yosuke Kurashima, Ph.D.		特任准教授	博士（医学）	倉　島　洋　介

To explore new avenues for mucosal vaccine development and immune-regulation, investigators have begun to employ novel adjuvants and targeting mucosal tissues and immune cells for vaccine delivery and elucidate the mechanisms of immune-regulation in the mucosal tissues. Despite recent advanced sciences, it remains to develop effective mucosal vaccines for human use. To this end, our main task is to define the effectiveness and safety of novel mucosal vaccines including adjuvant- and delivery system-development, and bring them from bench-top to practical applications.

1. Novel mucosal vaccine development for the induction of mucosal immunity in the aero-, digestive- and reproductive mucosa.

Kohtaro Fujihashi[1,2], Koichiro Suzuki[1], Rika Nakahashi[3], Yuya Murakami[1], Masao Uchida[1], Shiho Kurokawa[3], Kotomi Sugiura[3], Yoshikazu Yuki[3], and Hiroshi Kiyono[1-4]

[1]Division of Clinical Vaccinology, International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo, Tokyo 108-8639 Japan, [2]Department of Pediatric Dentistry, The University of Alabama at Birmingham, Birmingham, AL 35294-0007 USA [3]Department of Mucosal Immunology, IMSUT Distinguished Professor Unit, The Institute of Medical Science, The University of Tokyo, Tokyo 108-8639 JAPAN, [4]Department of Mucosal Immunology and Immunology, Graduate School of Medicine, Chiba University, Chiba, 260-8670, JAPAN

It has been shown that oral antigen (Ag) plus adjuvant delivery for induction of immunity, as opposed to nasal delivery, is an effective non-invasive route. Further, it is well-tolerated and avoids the possibility of Ag and /or adjuvant uptake into the olfactory tissues with subsequent entry into the central nervous system (CNS). However, oral vaccines require relatively large amounts of Ag and adjuvant and are exposed to the proteolytic enzymes and lower pH of the stomach. Considerably, their efficacy limits the mainly gastrointestinal mucosa. In this regard, it is essential to develop a new generation of oral adjuvants which elicit mucosal immunity in the entire mucosal surfaces including respiratory and reproductive tracts. In order to accomplish this goal, we planned to discover novel molecules which could use potential oral adjuvant for inducing global protective mucosal immunity by using a single-cell mRNA sequencing approach. We have successfully established several DNA libraries from nasopharyngeal-associated lymphoid tissues and Peyer's patches of naïve mice as well as mice given either oral or nasal vaccine. The sequence data have been analyzed using SHIROKANE supercomputer system and we have identified several unique molecules which preferentially upregulated in the NALT of mice given nasal vaccine when compared with those in Peyer's patches of mice given an oral vaccine. Our results showed that one of these molecules is indeed up-regulated in NALT and the reproductive tract. Thus, depletion of this molecule resulted in impaired antigen-specific IgA antibody responses in the reproductive tract of mice given the nasal vaccine. We are currently confirming the roles of other candidate molecules for the induction of mucosal tissue-specific immune responses by us-

ing gain and lost function approaches.

2. Dendritic cell-targeting Flt3 ligand and CpG ODN as nasal adjuvants induce oral pathogen-specific IgA antibody for the prevention of aspirate pneumonia

Kosuke Kataoka[1], Shigetada Kataoka[2], Kayo Koyanagi[1], Yoshiya Hashimoto[3], Tetsuro Miyake[1] and Kohtaro Fujihashi[4, 5]

[1]Departments of Preventive and Community Dentistry, Faculty of Dentistry, Osaka Dental University, Hirakata 573-1144, JAPAN, [2]Department of Oral and Molecular Microbiology, Graduate School of Dentistry, Osaka University, Suita 565-0871, Japan, [3]Department of Biomaterials, Faculty of Dentistry, Osaka Dental University, Hirakata 573-1144, Japan, [4]Division of Clinical Vaccinology, International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo, Tokyo 108-8639 JAPAN [5]Department of Pediatric Dentistry, The University of Alabama at Birmingham, Birmingham, AL 35294-0007 USA

Our previous studies showed that a combination of a DNA plasmid encoding Flt3 ligand (pFL) and CpG oligodeoxynucleotides 1826 (CpG ODN) (FL/CpG) as nasal adjuvant provoked antigen-specific immune responses. In this study, we investigated the efficacy of a nasal vaccine consisting of FimA as the structural subunit of *Porphyromonas gingivalis* fimbriae and FL/CpG for the induction of FimA-specific antibody (Ab) responses and their protective roles against nasal and lung infection by *P. gingivalis*, a keystone pathogen in the etiology of periodontal disease. C57BL/6 mice were nasally immunized with recombinant FimA (*r*FimA) plus FL/CpG three times at weekly intervals. As a control, mice were given nasal *r*FimA alone. Nasal washes and bronchoalveolar lavage fluid of mice given nasal *r*FimA plus FL/CpG resulted in increased levels of *r*FimA-specific secretory IgA and IgG Ab responses when compared with those in controls. Significantly increased numbers of CD8- or CD11b-expressing mature-type dendritic cells (DCs) were detected in the respiratory inductive and effector tissues of mice given *r*FimA plus FL/CpG. Additionally, significantly upregulated Th1/Th2-type cytokine responses by *r*FimA-stimulated $CD4^+$ T cells were noted in the respiratory effector tissues. When mice were challenged with live *P. gingivalis* via the nasal route, mice immunized nasally with *r*FimA plus FL/CpG inhibited *P. gingivalis* colonization in the nasal cavities and lungs. In contrast, controls failed to show protection. Of interest, when IgA-deficient mice given nasal *r*FimA plus FL/CpG were challenged with nasal *P. gingivalis*, the inhibition of bacterial colonization in the respiratory tracts was not seen. Taken together, these results show that nasal FL/CpG effectively enhanced DCs and provided balanced Th1- and Th2-type cytokine response-mediated *r*FimA-specific IgA protective immunity in the respiratory tract against *P. gingivalis*. A nasal administration with *r*FimA and FL/CpG could be a candidate for potent mucosal vaccines for the elimination of inhaled *P. gingivalis* in periodontal patients.

3. Orally desensitized mast cells form a regulatory network with Treg cells for the control of food allergy

Yoshihiro Takasato[1,2], Yosuke Kurashima[1,3-7], Masahiro Kiuchi[8], Kiyoshi Hirahara[8], Sayuri Murasaki[1,4], Fujimi Arai[1,4], Kumi Izawa[9], Ayako Kaitani[9], Kaoru Shimada[1,4], Yukari Saito[3], Shota Toyoshima[10], Miho Nakamura[1], Kumiko Fujisawa[1], Yoshimichi Okayama[10], Jun Kunisawa[4,7], Masato Kubo[11,12], Naoki Takemura[3,4,13], Satoshi Uematsu[3,4,14], Shizuo Akira[15,16], Jiro Kitaura[9], Takao Takahashi[2], Toshinori Nakayama[8], and Hiroshi Kiyono[1,4-6,8]

[1]Department of Mucosal Immunology, The University of Tokyo Distinguished Professor Unit, The Institute of Medical Science, The University of Tokyo, Tokyo, 108-8639, JAPAN, [2]Department of Pediatrics, Keio University School of Medicine, Tokyo, 160-8582, JAPAN, [3]Department of Innovative Medicine and Mucosal Immunology, Graduate School of Medicine, Chiba University, Chiba, 260-8670, JAPAN, [4]International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo, Tokyo 108–8639, JAPAN, [5]Division of Gastroenterology, Department of Medicine, CU-UCSD Center for Mucosal Immunology, Allergy and Vaccines (CU-UCSD cMAV), University of California, San Diego, CA 92093-0956, USA, [6]Institute for Global Prominent Research, Chiba University, Chiba, 260-8670, JAPAN, [7]Laboratory of Vaccine Materials, Center for Vaccine and Adjuvant Research and Laboratory of Gut Environmental System, National Institutes of Biomedical Innovation, Health and Nutrition, Osaka 567-0085, JAPAN, [8]Department of Immunology, Graduate School of Medicine, Chiba University, Chiba, 260-8670, Japan, [8]Atopy Research Center, Juntendo University Graduate School of Medicine, Tokyo, 113-8421, JAPAN, [9]Allergy and Immunology Research Project Team, Research Institute of Medical Science, Center for Allergy, Center for Medical Education, Nihon University School of Medicine, Tokyo, 173-8610, JAPAN, [10]Laboratory for Cytokine Regulation, RIKEN Center for Integrative Medical Sciences, Yokohama, Kanagawa 230-0045, JAPAN, [11]Division of Molecular Pathology, Research Institute for Biomedical Science, Tokyo University of Science, Chiba 278-0022, Japan, [12]Laboratory of Bioresponse Regulation, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamada-oka, Suita, Osaka, 565-0871 JAPAN, [13]Department of Immunology and Genomics, Osaka

City University Graduate School of Medicine, 1-4-3 Asahi-machi, Abeno-ku, Osaka 545-8585, JAPAN, [14]Laboratory of Host Defense, WPI Immunology Frontier Research Center, Osaka University, Osaka 565-0871, JAPAN, [15]Department of Host Defense, Research Institute for Microbial Diseases, Osaka University, Osaka 565-0871, JAPAN

Oral immunotherapy (OIT) is an effective approach to controlling food allergies. Although the detailed molecular and cellular mechanisms of OIT are unknown currently, they must be understood to advance the treatment of allergic diseases in general. To elucidate the mechanisms of OIT, especially during the immunological transition from desensitization to allergy regulation, we generated a clinical OIT murine model and used it to examine immunological features after OIT. We found that in mice that completed OIT successfully, desensitized mast cells (MCs) showed functionally beneficial alterations, such as increased expression of regulatory cytokines and enhanced expansion of regulatory T cells. Importantly, regulatory-T-cell-mediated inhibition of allergic responses was decreased in mice in which desensitized MCs were depleted during OIT. Collectively, these findings show that the desensitization process modulates the activation of MCs, leading directly to enhanced induction of regulatory-T-cell expansion and promotion of clinical allergic unresponsiveness. Our results suggest that efficiently inducing regulatory MCs is a novel strategy for the treatment of allergic diseases.

4. Pancreatic glycoprotein 2 is a first line of defense for mucosal protection in intestinal inflammation.

Yosuke Kurashima[1,2,3,4,5,18], Takaaki Kigoshi [1,6,18], Sayuri Murasaki[1,3], Fujimi Arai[1,3], Kaoru Shimada[1,3], Natsumi Seki[7,8], Yun-Gi Kim[8], Koji Hase [7,3], Hiroshi Ohno[9,10,11], Kazuya Kawano[9], Hiroshi Ashida[12,13], Toshihiko Suzuki[12], Masako Morimoto[2], Yukari Saito[2], Ai Sasou[1,3], Yuki Goda[1,3], Yoshikazu Yuki[1,3], Yutaka Inagaki[14], Hideki Iijima[15], Wataru Suda[16], Masahira Hattori[16,17], and Hiroshi Kiyono [1,3,4,5]

[1]Department of Mucosal Immunology, The University of Tokyo Distinguished Professor Unit, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan, [2]Department of Innovative Medicine, Graduate School of Medicine, Chiba University, Chiba, Japan, [3] International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan, [4]Division of Gastroenterology, Department of Medicine, CU-UCSD Center for Mucosal Immunology, Allergy and Vaccines, University of California, San Diego, CA, USA, [5] Mucosal Immunology and Allergy Therapeutics, Institute for Global Prominent Research, Graduate School of Medicine, Chiba University, Chiba, Japan, [6]Department of Pediatrics, Graduate School of Medicine, Tohoku University, Miyagi, Japan, [7]Division of Biochemistry, Graduate School of Pharmacological Science, Keio University, Tokyo, Japan, [8] Research Center for Drug Discovery, Faculty of Pharmacy, Keio University, Tokyo, Japan, [9] Laboratory for Intestinal Ecosystem, RIKEN Center for Integrative Medical Sciences, Kanagawa, Japan, [10]Division of Immunobiology, Department of Medical Life Science, Graduate School of Medical Life Science, Yokohama City University, Kanagawa, Japan, [11]Intestinal Microbiota Project, Kanagawa Institute of Industrial Science and Technology, Kanagawa, Japan, [12]Department of Bacterial Infection and Host Response, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan, [13]Medical Mycology Research Center, Chiba University, Chiba, Japan, [14]Center for Matrix Biology and Medicine, Graduate School of Medicine, Tokai University, Kanagawa, Japan, [15]Department of Gastroenterology and Hepatology, Graduate School of Medicine, Osaka University, Osaka, Japan, [16]Laboratory for Microbiome Sciences, RIKEN Center for Integrative Medical Sciences, Kanagawa, Japan, [17]Graduate School of Advanced Science and Engineering, Waseda University, Tokyo, Japan

Increases in adhesive and invasive commensal bacteria, such as *Escherichia coli*, and subsequent disruption of the epithelial barrier are implicated in the pathogenesis of inflammatory bowel disease (IBD). However, the protective systems against such barrier disruption are not fully understood. Here, we show that secretion of luminal glycoprotein 2 (GP2) from pancreatic acinar cells is induced in a TNF-dependent manner in mice with chemically induced colitis. Fecal GP2 concentration is also increased in Crohn's disease patients. Furthermore, pancreas-specific GP2-deficient colitis mice have more severe intestinal inflammation and a larger mucosal *E. coli* population than do intact mice, indicating that digestive-tract GP2 binds commensal *E. coli*, preventing epithelial attachment and penetration. Thus, the pancreas-intestinal barrier axis and pancreatic GP2 are important as the first line of defense against adhesive and invasive commensal bacteria during intestinal inflammation.

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International Research and Development Center for Mucosal Vaccines

Division of Mucosal Vaccines
粘膜ワクチン学分野

Professor	Ken J Ishii, M.D., Ph.D.	教授	博士(医学)	石 井	健
Visiting Professor	Jun Kunisawa, Ph.D.	客員教授	博士(薬学)	國 澤	純
Visiting Associate Professor	Tomonori Nochi, Ph.D.	客員准教授	博士(農学)	野 地 智	法
Project Senior Assistant Professor	Rika Nakahashi, Ph.D.	特任講師	博士(医学)	中 橋 理	佳

Mucosal vaccine is a prospective strategy for the vaccine development against pathogens invading through mucosal tissues. We have examined the immunological functions of commensal and pathogenic microorganisms as well as diets and applied them to the development of adjuvants and antigen delivery for the efficient immune responses against mucosal vaccines. These findings also could be extended to the development of mucosal immunotherapy against allergic, inflammatory, and infectious diseases.

1. Application of *Alcaligenes* LPS/lipid A for an effective and safe adjuvant for respiratory infection vaccines

Koji Hosomi[1], Yunru Wang[1,2], Zilai Liu[1,2], Ken Yoshii[1,3], Atsushi Shimoyama[4], Takahiro Nagatake[1], Azusa Saika[1], Yoko Tojima[1], Mari Furuta[1], Yukari Fujimoto[5], Koichi Fukase[4], Hiroshi Kiyono[6-9], Jun Kunisawa[1-4,6,10-12]

[1] Laboratory of Vaccine Materials, Center for Vaccine and Adjuvant Research and Laboratory of Gut Environmental System, National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN), [2]Graduate School of Pharmaceutical Sciences, Osaka University, [3]Graduate School of Medicine, Osaka University, [4]Graduate School of Science, Osaka University, [5]Faculty of Science and Technology, Keio University, [6]International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo, [7]Division of Gastroenterology, Department of Medicine, University of California San Diego (UCSD), [8]Chiba University (CU)-UCSD Center for Mucosal Immunology, Allergy and Vaccines, [9]Department of Immunology, Graduate School of Medicine, Chiba University, [10]Faculty of Science and Engineering, Waseda University, [11]Graduate School of Dentistry, Osaka University, [12]Department of Microbiology and Immunology, Kobe University Graduate School of Medicine

Lymphoid-tissue–resident commensal bacteria (LRCs), including *Alcaligenes faecalis*, colonize within dendritic cells (DCs) in intestinal lymphoid tissue including the Peyer's patches (PPs) of mammals. LRCs modulate the host immune system to promote intestinal IgA antibody responses. Indeed, we previously demonstrated that *A. faecalis* activates DCs to produce IL-6, an IgA production-enhancing cytokine, through the weak agonistic activity of its lipopolysaccharide (LPS) against toll-like receptor (TLR) 4 together with low inflammatory activity. Unique characteristics of *Alcaligenes* LPS, which moderately activate host immune responses, promoted us to be examined for application as a vaccine adjuvant. We previously reported that *Alcaligenes* LPS promoted antigen-specific immune responses including IgG antibody and Th17 responses without excessive inflammation.

Here, we examined the efficacy of *Alcaligenes* LPS as a nasal vaccine adjuvant. Nasal immunization with ovalbumin (OVA) plus *Alcaligenes* LPS induced follicular T helper cells and germinal center formation in

the nasopharynx-associated lymphoid tissue (NALT) and cervical lymph nodes (CLNs), and consequently enhanced OVA-specific IgA and IgG responses in the respiratory tract and serum. In addition, nasal immunization with OVA plus *Alcaligenes* LPS induced OVA-specific T cells producing IL-17 and/or IL-10 and, whereas nasal immunization with OVA plus cholera toxin (CT), a gold standard experimental mucosal adjuvant, induced OVA-specific T cells producing IFN-γ and IL-17, which are recognized as pathogenic type of Th17 cells. Consistently, nasal immunization with OVA plus CT, but not *Alcaligenes* LPS, led to increased numbers of neutrophils and eosinophils in the nasal cavity. Thus, *Alcaligenes* LPS showed effective adjuvant activity to nasal vaccination without activation of inflammatory cascade after nasal administration.

To extend the possibility of *Alcaligenes* LPS as a vaccine adjuvant, together with the characterization of the structure of lipid A, the biologically active site of LPS, and establishment of its chemical synthesis method, we applied it to the vaccine adjuvant. Like LPS, our previous studies showed that chemically synthesized *Alcaligenes* lipid A showed effective adjuvant activity to induce Th17 polarization and to enhance systemic IgG and respiratory IgA responses to T cell dependent antigens such as OVA and pneumococcal surface protein A mediated by DC activation, which was sufficient for protection against for *Streptococcus pneumoniae* infection. In contrast, our recent study indicated that when immunized with *Haemophilus* influenzae B conjugate vaccine that contains capsular polysaccharide polyribosyl ribitol phosphate (PRP), a T cell independent antigen, *Alcaligenes* lipid A enhance PRP-specific IgG antibody production but not Th17 response. In addition, *in vitro* coculture with *Alcaligenes* lipid A promoted significant proliferation of and enhanced antibody production by B cells. Together, *Alcaligenes* lipid A has adjuvant activity to both T cell-dependent and -independent antigens, but there appear to be difference in T cell responses and mechanisms of action such as targeting cells.

2. Intestinal microbe-dependent and -independent metabolism of ω3 lipids for the control of allergic and inflammatory diseases

Takahiro Nagatake[1], Azusa Saika[1], Shigenobu Kishino[2], Emiko Urano[3], Haruka Murakami[4], Nahoko Kitamura[2], Kana Konishi[4], Harumi Ohno[4], Prabha Tiwari[1], Sakiko Morimoto[1], Eri Node[1], Jun Adachi[5], Yuichi Abe[5,6], Junko Isoyama[5], Kento Sawane[1,7], Tetsuya Honda[8,9], Asuka Inoue[10], Akiharu Uwamizu[10,11], Takashi Matsuzaka[12,13], Yoichi Miyamoto[14], So-ichiro Hirata[1,15], Yuki Shibata[1,7], Koji Hosomi[1], Ayu Matsunaga[1,16], Hitoshi Shimano[12], Makoto Arita[17-19], Junken Aoki[10,11], Masahiro Oka[14], Akira Matsutani[20], Takeshi Tomonaga[5], Kenji Kabashima[8], Motohiko Miyachi[4], Yasuhiro Yasutomi[3], Jun Ogawa[2], and Jun Kunisawa[1,7,15,21-23]

[1]Laboratory of Vaccine Materials, Center for Vaccine and Adjuvant Research and Laboratory of Gut Environmental System, National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN), [2]Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, [3]Laboratory of Immunoregulation and Vaccine Research, Tsukuba Primate Research Center, NIBIOHN, [4]Department of Physical Activity Research, NIBIOHN, [5]Laboratory of Proteome Research and Laboratory of Proteomics for Drug Discovery, NIBIOHN, [6]Division of Molecular Diagnosis, Aichi Cancer Center Research Institute, [7]Graduate School of Pharmaceutical Sciences, Osaka University, [8]Department of Dermatology, Kyoto University Graduate School of Medicine, [9]Department of Dermatology, Hamamatsu University School of Medicine, [10]Department of Molecular and Cellular Biochemistry, Graduate School of Pharmaceutical Sciences, Tohoku University, [11]Graduate School of Pharmaceutical Sciences, The University of Tokyo, [12]Department of Endocrinology and Metabolism, Faculty of Medicine, University of Tsukuba, [13]Transborder Medical Research Center, University of Tsukuba, [14]Laboratory of Nuclear Transport Dynamics, NIBIOHN, [15]Department of Microbiology and Immunology, Kobe University Graduate School of Medicine, [16]Department of Food and Life Science, School of Life and Environmental Science, Azabu University, [17]Division of Physiological Chemistry and Metabolism, Keio University Faculty of Pharmacy, [18]Laboratory for Metabolomics, RIKEN Center for Integrative Medical Sciences, [19]Cellular and Molecular Epigenetics Laboratory, Graduate School of Medical Life Science, Yokohama City University, [20]Department of Internal Medicine, Shunan City Shin-nanyo Hospital, [21]International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo, [22]Graduate School of Medicine, Graduate School of Dentistry, Graduate School of Science, Osaka University, [23]Research Organization for Nano and Life Innovation, Waseda University.

Immune system is regulated by various environmental factors, including dietary lipids and intestinal microbiota. We previously found that dietary intake of linseed oil, rich in ω3 α-linolenic acid, led to the amelioration of allergic responses in the gut and nasal mucosa as well as infant skin through the metabolic conversion of α-linolenic acid into anti-allergy and anti-inflammatory lipid mediators. Here, we extended our point of view by showing that the fatty acid metabolism is conducted not only by mammalian-dependent but also by intestinal microbe-dependent pathways.

In mammalian-dependent pathways, we recently found that 12-hydroxyeicosapentaenoic acid (12-

HEPE), which is the dominant ω3 fatty acid metabolite in the skin of mice fed with linseed oil, inhibited the inflammation associated with allergic dermatitis by inhibiting neutrophil infiltration. 12-HEPE downregulated the expression of neutrophil chemoattractants, *CXCL1* and *CXCL2*, on keratinocytes in retinoid X receptor (RXR) α-dependent manner. We further found that dietary linseed oil inhibited the pathogenesis of high-fat diet-induced atherosclerosis via production of 12-HEPE. Indeed, 12-HEPE acted on macrophages and inhibited oxidized low-density lipoprotein-induced foam cell formation.

In intestinal microbe-dependent pathway, we found that α-linolenic acid was converted to αKetoA via αHYA in specific-pathogen free but not germ-free mice. αKetoA but not αHYA showed potent anti-inflammatory properties through a peroxisome proliferator-activated receptor (PPAR) γ-dependent fashion and ameliorated hapten-induced contact hypersensitivity by inhibiting the development of inducible skin-associated lymphoid tissue through suppression of chemokine secretion from macrophages and inhibition of NF-κB activation in mice and cynomolgus macaques. Administering αKetoA also improved diabetic glucose intolerance by inhibiting adipose tissue inflammation and fibrosis through decreased macrophage infiltration in adipose tissues and altering macrophage M1/M2 polarization in mice fed a high-fat diet. These results collectively demonstrate that dietary ω3 lipids exert various features of immunoregulatory activities through conversion to unique lipid metabolites by mammalian cells and intestinal microbes.

3. Gut microorganisms induce Peyer's patch-dependent maternal IgA production in milk

Katsumi Usami[1], Kanae Niimi[1], Ayumi Matsuo[1], Yoshihisa Suyama[1], Yoshifumi Sakai[1], Shintaro Sato[2], Kohtaro Fujihashi[3], Hiroshi Kiyono[4], Saeka Uchino[1], Mutsumi Furukawa[1], Jahidul Islam[1], Kaori Ito[1], Taiki Moriya[5], Yutaka Kusumoto[5], Michio Tomura[5], Russell C. Hovey[6], Junichi Sugawara[7], Hiroshi Yoneyama[1], Haruki Kitazawa[1], Kouichi Watanabe[1], Hisashi Aso[1], Tomonori Nochi[8].

[1] International Education and Research Center for Food and Agricultural Immunology, Graduate School of Agricultural Science, Tohoku University, [2] Mucosal Vaccine Project, BIKEN Innovative Vaccine Research Alliance Laboratories, Research Institute for Microbial Diseases, Osaka University; Development of Immunology and Genomics, Graduate School of Medicine, Osaka City University; Division of Clinical Vaccinology, International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo, [3] Division of Clinical Vaccinology, International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo; Department of Pediatric Dentistry, The University of Alabama at Birmingham, [4] IMSUT Distinguished Professor Unit, Division of Mucosal Immunology, The Institute of Medical Science, The University of Tokyo; Department of Immunology, Graduate School of Medicine, Chiba University; Department of Medicine, School of Medicine and CU-UCSD Center for Mucosal Immunology, Allergy & Vaccines, University of California, San Diego, [5] Laboratory of Immunology, Faculty of Pharmacy, Osaka Ohtani University, [6] Department of Animal Science, University of California, Davis, Davis, [7] Tohoku Medical Megabank Organization, Tohoku University, [8] International Education and Research Center for Food and Agricultural Immunology, Graduate School of Agricultural Science, Tohoku University; Division of Mucosal Vaccines, International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo.

An approach to increasing the quality of breastfeeding brings mammalian health across generations. The inter-organ network among distinct tissues has been implicated in maintaining essential behaviors including breastfeeding; however, most of the details are unknown. Here, we describe that the role of Peyer's patches (PPs), which are a secondary lymphoid tissue in the small intestine, is essential for breastfeeding. Specifically, PPs play a key role as a source of plasma cells recruited from the mammary gland to produce maternal IgA, which is transferred from mother to the offspring via breastfeeding. A more significant advance in this study is that *Bacteroides acidifaciens* and *Prevotella buccalis*, both of which belong to Bacteroidales, are identified as essential bacteria in the gastrointestinal tract for stimulating the immune functions in PPs to produce maternal IgA in milk. Our results provide significant insights into the development of novel strategies that can be used for transferring sufficient amounts of maternal IgA to the next generation via breastfeeding.

4. Development of cationic nanogel-based nasal vaccines for various infectious diseases

Rika Nakahashi[1,2], Yoshikazu Yuki[1,3], Hiromi Mori[1], Shingo Umemoto[1,4], Yohei Uchida[1], Tomoyuki Yamanoue[1], Tomonori Machita[1], Shiho Kurokawa[1], Kotomi Sugiura[1], Kohtaro Fujihashi[5], Hiroshi Kiyono[2,3,6,7]

[1] International Research and Development Center for Mucosal Vaccine, The Institute of Medical Science, The University of Tokyo, [2] Department of Mucosal Immunology, IMSUT Distinguished Professor Unit, The Institute of Medical Science, The University of Tokyo, [3] HanaVax Inc., [4] Faculty of Medicine, Department of Otorhinolaryngology and Head and Neck Surgery, Oita University, [5] Research and De-

velopment Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo, [6] Mucosal Immunology and Allergy Therapeutics, Institute for Global Prominent Research, Chiba University, [7] Division of Gastroenterology, Department of Medicine, School of Medicine, CU-UCSD Center for Mucosal Immunology, Allergy and Vaccines, University of California, San Diego.

Nasal vaccines not only induce an antigen-specific systemic immune response but induce antigen-specific secretory IgA at the mucosal site to which the vaccine is administered, thereby blocking the invasion of foreign microorganisms into the host. Furthermore, it induces an antigen-specific immune response also at the mucosal surface distant from the administration site such as genital tract through lymphocyte homing mechanisms. Based on these advantages of nasal vaccine, we are developing nasal vaccines for various infectious diseases by using a cationic type of cholesteryl group-containing pullulan nanogel (cCHP nanogel), a novel drug delivery system for nasal vaccine. Previously, we have demonstrated the efficacy of cCHP nanogel-based nasal vaccines for various respiratory infectious diseases such as Pneumonia, non-typeable *Haemophilus influenzae*, RSV, Tuberculosis, or sexually transmitted disease such as cervical cancer caused by human papilloma viruses by using mouse model. Furthermore, based on the effectiveness of the cCHP nanogel nasal vaccination system, we have started the development of nasal vaccine for COVID-19 which the pandemic is ongoing with no sign of convergence. We aim to examine the optimal nasal vaccine formulation by testing the different antigen candidates and/or mucosal adjuvants.

5. Comparative analysis on whole-genome and proteomics between the next seed bank and the original master seed bank of a rice-based oral cholera vaccine MucoRice-CTB 51A line.

Ai Sasou[1], Yoshikazu Yuki[1], Ayaka Honma[1], Kotomi Sugiura[1], Koji Kashima[2], Hiroko Kozuka-Hata[3], Masanori Nojima[4], Masaaki Oyama[3], Shiho Kurokawa[1], Shinichi Maruyama[2], Masaharu Kuroda[5], Shinjiro Tanoue[6], Narushi Takamatsu[6], Kohtaro Fujihashi[7], Eiji Goto[8] and Hiroshi Kiyono[1,9,10]

[1] Division of Mucosal Immunology, IMSUT Distinguished Professor Unit, The Institute of Medical Science, The University of Tokyo, [2] Asahi Kogyosha Co., Ltd., [3] Medical Proteomics Laboratory, The Institute of Medical Science, The University of Tokyo, [4] Center for Translational Research, IMSUT Hospital, The Institute of Medical Science, The University of Tokyo, [5] Crop Development Division, NARO Agriculture Research Center, [6] Astellas Pharma Inc., Tokyo, [7] Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo, [8] Faculty of Horticulture, Graduate School of Horticulture, Chiba University, [9] Department of Immunology, Graduate School of Medicine, Chiba University, [10] Chiba University-University of California San Diego Center for Mucosal Immunology, Allergy, and Vaccine, Division of Gastroenterology, Department of Medicine, University of California, San Diego.

We have previously developed a rice-based oral vaccine against cholera diarrhea, MucoRice-CTB, based on a plant genetic engineering technology. Then we succeeded in producing the selection marker-free MucoRice-CTB line 51A. In the line 51A, three copies of the cholera toxin B subunit (CTB) gene and two copies of the RNAi cassette were inserted into the rice genome by using *Agrobacterium*-mediated co-transformation. The sequence and position of the transgenes on rice chromosomes 3 and 12 were determined and the expression of α-amylase/trypsin inhibitor, a major allergenic protein in rice, was lower in the 51A line than that in the wild-type rice. The 51A line was then self-pollinated for five generations to fix the transgenes, and the sixth generation of seeds produced by T5 plants was defined as the master seed bank (MSB). The T6 plants were grown from some seeds of MSB, then T7 seeds were produced and defined as a next seed bank (NSB) after the self-pollination. The whole genome and proteome were analyzed and compared between MSB and NSB. A clustering analysis indicated that the DNA sequences of the transgenes were identical between MSB and NSB, and no functionally important mutations (SNPs, translocations, deletions, and inversions of gene regions on chromosomes) were detected in three samples of each bank. Furthermore, shotgun MS/MS analysis of salt-soluble proteins in NSB and MSB samples showed no significant differences in protein levels were detected. In addition, no differences in the expression patterns of storage proteins and CTB in mature seeds of NSB and MSB were observed by immunofluorescence microscopy. Since any considerable differences were not observed between NSB and MSB samples, we conclude that NSB can be used to replace MSB in future.

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International Research and Development Center for Mucosal Vaccines

Division of Mucosal Symbiosis
粘膜共生学分野

Project Associate Professor	Yoshiyuki Goto, Ph.D.		特任准教授	博士(医学)	後 藤 義 幸
Professor	Tetsuro Matano, M.D., Ph.D.		委嘱教授	博士(医学)	俣 野 哲 朗

The gastrointestinal tract is a unique organ that is constitutively exposed by various antigens, including commensal microbiota. In order to create a symbiotic environment for non-pathogenic luminal microorganisms, epithelial cells (ECs) and immune cells cooperatively establish homeostasis of the intestinal microenvironment. We aim to identify the mechanisms of epithelial α1, 2-fucosylation, one of the symbiotic factors between host and microbiota, and uncover the role of ECs-immune cell network in the establishment of intestinal homeostasis. We also aim to understand host-microbe as well as microbe-microbe interaction in the gut.

1. Innate immune lymphocytes govern intestinal epithelial α1, 2-fucosylation

Yoshiyuki Goto[1,2], and Hiroshi Kiyono[1,3-5]

[1] International Research and Development Center for Mucosal Vaccine, Institute for Medical Science, The University of Tokyo, [2] Division of Molecular Immunology, Medical Mycology Research Center, Chiba University, [3] Division of Innate immune regulation, [3] Mucosal Immunology and Allergy Therapeutics, Institute for Global Prominent Research, Chiba University, [4] Division of Gastroenterology, Department of Medicine, School of Medicine, CU-UCSD Center for Mucosal Immunology, Allergy and Vaccines, University of California, San Diego, [5] Division of Mucosal Immunology, IMSUT Distinguished Professor Unit, The Institute of Medical Science, The University of Tokyo.

α1, 2-fucosyl linkages located to terminal carbohydrate moiety expressed on intestinal epithelial cells are catalyzed by fucosyltransferase 2 (Fut2). Epithelial α1, 2-fucose is one of the symbiotic factors that mediate host-microbiota interaction. For example, epithelial α1, 2-fucose is utilized as a dietary carbohydrate by various symbiotic bacteria such as *Bacteroides*. Therefore, disruption of Fut2 leads to dysbiosis both in mice and humans and is predisposed to the development of inflammatory diseases such as Crohn's disease. Despite the importance of intestinal and systemic homeostasis, the molecular and cellular mechanisms of the induction of epithelial Fut2, and subsequent α1, 2-fucosylation remain unknown. We found that group 3 innate lymphoid cells (ILC3) are critical inducers of intestinal epithelial Fut2 expression and fucosylation mediated by the production of interleukin 22 and lymphotoxin from ILC3 in a commensal bacteria-dependent and -independent manner, respectively. Fut2-deficient mice are susceptible to infection by pathogenic microorganisms. These data unveil a novel function of ILC3 in creating the appropriate symbiotic environment and protective platform against pathogenic microorganisms through regulating the epithelial α1, 2-fucosylation.

2. Commensal microbiota prevent fungi from colonizing the gastrointestinal tract.

Yoshiyuki Goto[1,2], Tetsuro Matano[1]

[1] Division of Mucosal symbiosis, International Research and Development Center for Mucosal Vaccine, Institute for Medical Science, The University of

Tokyo, [2] Division of Molecular Immunology, Medical Mycology Research Center, Chiba University.

Intestinal epithelial cells are the first line of defense against infection by pathogenic microorganisms. *Candida albicans* are one of the commensal fungi that reside in the mucosal surface, including the gastrointestinal tract. However, *C. albicans* also have been reported to exert pathogenic effects in the immunocompromised host and expand to the systemic compartments, called invasive candidiasis. Invasive candidiasis triggered by *C. albicans* colonization in the gut is one of the serious infectious diseases in the world. So far, it is unclear what kind of factors regulate *C. albicans* colonization in the gut. To investigate this, we focused on the role of commensal bacteria against colonization by *C. albicans*. We found that germfree and several antibiotic-treated mice allow *C. albicans* colonization in the gut. Furthermore, oral administration of feces isolated from normal mice excluded *C. albicans* from the gut. This data suggests that commensal bacteria prevent the colonization of *C. albicans* in the gut, and commensal bacteria may be a novel therapeutic target for protection against *C. albicans* infection.

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Center for Gene and Cell Therapy

Division of Molecular and Medical Genetics
分子遺伝医学分野

Professor	Takashi Okada, M.D., Ph.D.	教　授	博士(医学)	岡　田　尚　巳
Associate Professor	Naoya Uchida, M.D., Ph.D.	准教授	博士(医学)	内　田　直　也
Assistant Professor	Yuji Tsunekawa, Ph.D.	助　教	博士(医学)	恒　川　雄　二
Project Assistant Professor	Hiromi Hayashita-Kinoh, Ph.D.	特任助教	博士(医学)	喜　納　裕　美
Project Assistant Professor	Yasunari Matsuzaka, Ph.D.	特任助教	博士(医学)	松　坂　恭　成
Project Assistant Professor	Yuko Nitahara-Kasahara, Ph.D.	特任助教	博士(医学)	笠　原　優　子

The Division of Molecular and Medical Genetics (DMMG) was generated in 2020, and we focus on the development of gene-addition/editing therapy including viral vector preparation and purification, adeno-associated virus (AAV) vector-mediated gene therapy for Duchenne muscular dystrophy (DMD), and hematopoietic stem cell (HSC)-targeted gene therapy with lentiviral vectors. Our basic and translational efforts can allow producing new genetic therapies for various neuromuscular, hematologic, and metabolic diseases.

Advanced methods for AAV vector preparation and purification for *in vivo* gene therapy

AAV vectors are being used for the development of *in vivo* gene therapy to cure various hereditary diseases including DMD. However, a detailed qualitative analysis of AAV vectors is required for *in vivo* gene therapy according to the recent safety regulation. We are conducting various research projects for (1) generation of an efficient and practical cell line to prepare AAV vectors, (2) improvement of large-scale preparation methods for AAV vectors, (3) development of analysis (electron microscopy) and purification (density-gradient ultracentrifugation) methods among empty-, partial-, and full-genome containing particles in AAV vectors, and (4) evaluation of glycosylation on AAV capsids. Ultimately, we are planning to generate AAV vectors at the GMP level for a gene therapy trial.

Evaluation of exosome-mediated delivery of AAV vectors

Exosomes are membrane-bound extracellular vesicles, composed of the cell membrane and endosomal compartments. Recently, AAV vectors were reported to be packaged to exosomes (named vexosomes) possibly resulting in high-efficiency transduction, specific delivery, and resistance to neutralizing antibodies. We are going to generate and analyze exosome-mediated AAV vectors to evaluate these potential functions.

Innovation in AAV vector toward gene and cell therapies to treat neuromuscular diseases

DMD is caused by a mutation of the dystrophin gene in the X chromosome and induces progressive weakness and atrophy of both skeletal and heart muscles, thus resulting in motor dysfunction, cardiomyopathy, respiratory failure, and early mortality. The current FDA-approved treatments, such as a corticosteroid (Deflazacort) and antisense oligonucleotides (Eteplirsen and Golodirsen), seem to be insufficient for the cure in DMD. Therefore, we are developing AAV vector-based *in vivo* gene therapy which is potentially curative for DMD by adding a micro-dystrophin gene or an exon-skipping molecule. We have demonstrated an improvement of heart functions by

a micro-dystrophin gene delivery with systemic injection of an AAV vector in a DMD model dog, and co-injection of mesenchymal stem cells (MSCs) allowed for elongation of transgene expression as well as reduction of AAV-vector dose. Now, we are going to perform a pilot trial of MSC-based cell therapy in DMD. In addition, we are planning an AAV vector-based gene therapy trial with co-injection of MSCs.

Development of HSC-targeted gene therapy with lentiviral vectors

HSC-targeted gene therapy with lentiviral vectors can cure various inherited diseases, including immunodeficiencies, hemoglobinopathies, and metabolic diseases. In HSC gene therapy, patient CD34+ HSCs are harvested and genetically modified with lentiviral vectors to deliver a therapeutic gene, followed by HSC transplantation back to the patient. This strategy can be applicable for most patients due to no requirement of the histocompatible donor, and it allows for a one-time cure of diseases. We are planning to develop HSC gene therapies for various genetic diseases in collaboration with National Center for Child Health and Development.

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Center for Gene & Cell Therapy
遺伝子・細胞治療センター

Director/Professor	Takashi Okada, M.D., Ph.D.		センター長／教授	博士(医学)	岡 田 尚 巳
Professor	Arinobu Tojo, M.D., D.M.Sc.		教 授	医学博士	東 條 有 伸
Professor	Tomoki Todo, M.D., Ph.D.		教 授	博士(医学)	藤 堂 具 紀
Professor	Toshio Kitamura, M.D., D.M.Sc.		教 授	医学博士	北 村 俊 雄
Professor	Fumitaka Nagamura, M.D., D.M.Sc.		教 授	博士(医学)	長 村 文 孝
Invited Professor	Koji Tamada, M.D., Ph.D.		教授(委嘱)	博士(医学)	玉 田 耕 治
Project Professor	Hideaki Tahara, M.D., D.M.Sc.		特任教授	医学博士	田 原 秀 晃
Project Professor	Satoshi Takahashi, M.D., D.M.Sc.		特任教授	博士(医学)	高 橋 聡
Visiting Professor	Shin-ichi Muramatsu, M.D., Ph.D.		客員教授	博士(医学)	村 松 慎 一
Associate Professor	Tokiko Nagamura-Inoue, M.D., Ph.D.		准教授	博士(医学)	長 村 登紀子
Project Associate Professor	Hiroaki Uchida, M.D., Ph.D.		特任准教授	博士(医学)	内 田 宏 昭

The Center for Gene & Cell Therapy (CGCT) was generated in 2014 at IMSUT hospital, playing a crucial role in hematopoietic stem cell (HSC) transplantation and gene therapy. We focus on the development of gene and cell therapy, regenerative therapy, and ethical, legal, and social implications (ELSI), including oncolytic virus and engineered T-cell therapies for malignancies, as well as AAV vector-mediated and HSC-targeted gene therapy for hereditary diseases.

Initiation of gene and cell therapy trials in hemophilia and Parkinson disease

We have generated a consortium including CGCT and Center for Stem Cell Biology and Regenerative Medicine in 2021 to develop a research center for gene and cell therapy, regenerative therapy, and ELSI research. In this consortium, we started a combined meeting that provided a seminar regarding advances of HSC-targeted gene-addition/editing therapy. Currently, we are considering gene therapy trials for hemophilia B as well as Parkinson's disease. Hemophilia B is a bleeding disorder, caused by a factor IX deficiency with a genetic mutation. Recombinant factor IX replacement therapy and bispecific antibody therapy are effective; however, they must be continued for a life-long with a high cost. AAV vector-mediated *in vivo* gene therapy is curative for the long-term in Hemophilia B by adding a therapeutic factor IX gene. In contrast, Parkinson's disease is a brain disorder caused by a loss of dopamine-producing cells in the brain. L-dopa treatment can reduce the symptoms of Parkinson's disease; however, it should be continued for a life-long, and the disease remains progressive. AAV vector-mediated *in situ* gene therapy can allow for phenotypic correction in Parkinson's disease by adding a neuroprotective molecule glial cell line-derived neurotrophic factor (GDNF). Both gene therapies should be promising candidates to develop a curative strategy.

Development of an oncolytic virus therapy for malignant glioma

Dr. Todo's group in the CGCT developed an oncolytic herpes simplex virus type 1 (G47Δ), designed to replicate only in cancer cells. Dr. Todo's group started a phase II, an investigator-initiated clinical trial for glioblastoma in 2015. In the intermediate analysis, the 1-year survival rate after G47Δ therapy (92.3%) was much higher than the preset control value (15%) based on a meta-analysis. Adverse events included fever, vomiting, lymphopenia, and nausea. Based on the high efficacy and safety shown in this phase II

study, G47Δ has been approved as a new drug for malignant glioma, and it's the first oncolytic virus product in Japan.

Running of the IMSUT-HLC Cell Processing Facility

Dr. Tokiko Nagamura has developed a new IMSUT-HLC Cell Processing Facility at the GCTP level in 2021 to generate and store cord blood-derived mesenchymal stem cells (MSCs) for immunosuppressive therapy in graft-versus-host disease. In addition, the IMSUT-HLC is utilized for cell manipulation in future clinical trials of gene and cell therapies as well as regenerative therapy in the CGCT.

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Laboratory Animal Research Center

Division of Animal Genetics
先進動物ゲノム研究分野

Professor	Tomoji Mashimo, Ph.D.	教　授	博士(人間・環境学)	真　下　知　士
Senior Assistant Professor	Kazuto Yoshimi, Ph.D.	講　師	博士(医科学)	吉　見　一　人
Assistant Professor	Saeko Ishida, D.V.M., Ph.D.	助　教	博士(医学)	石　田　紗恵子
Project Assistant Professor,	Tomoaki Fujii, Ph.D.	特任助教	博士(理学)	藤　井　智　明

Genome engineering technologies achieve a "revolution" in life and medical science. These techniques allow us to manipulate genes of interest for several purposes. Using those technologies, we have developed many useful strains in mice and rats. We are now focusing on generating "humanized animals" or "immunodeficient animals". These valuable animals can be used for xenotransplantation of human cells/tissues including human iPS cells. We are also developing therapeutic strategies such as cell therapy and gene therapy with genome editing tools.

Generation of several severe combined immunodeficiency rats

Jinxi Wang, Yoshiki Miyasaka[1], Kousuke Hattori, Miho Hoshi, Alejandro Soto-Gutierrez[2], Kazuki Takeishi[3], Kazuto Yoshimi, Saeko Ishida, and Tomoji Mashimo

1, Institute of Experimental Animal Sciences, Graduate School of Medicine, Osaka University, Osaka 565-0871, Japan

2, Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

3, Department of Pathology, University of Pittsburgh, Pittsburgh, PA, USA

The immunodeficiency animals are valuable experimental models, not only in the studies of immunodeficiency related diseases, they also have good performances in the application of grafting various tissues. Therefore, the immunodeficiency animals have been widely applied in generation of humanize animals, regeneration medical, tumor researches, etc. By utilizing the CRISPR/Cas9 genome editing tool, we generated a Severe Combined Immunodeficiency (SCID) rat model, which carry homozygous mutation in both Il2rg and Rag2 gene. These combined mutations caused the retard of both T cell and B cell development, as well as the deficiency of functional NK cells and cytokines secretion, providing favorable in vivo environment for the subsistence and proliferation of exogenous cells or tissues. Other than the immunodeficiency animals that generated by combining the mutations from different rat strains, our SCID rats have a clear genetic background of F344 rats. Our SCID rats has been set up a Bio-recourse project, and provided to institutes and researchers all over the world. In the following studies, we devote to modifying other genes in these SCID rats, to improve the efficiency of xenograft and alleviate acute xenogeneic graft-versus-host disease (GVHD) in the recipient SCID rats.

Development strategies of effective treatment for human cancers with CRISPR-Cas3 system

Tomoaki Fujii, Jinxi Wang, Ken Mochizuki, Kazuto Yoshimi, and Tomoji Mashimo

The genome editing system could be a powerful

genetic tools for development of human gene therapy around the world. However, CRISPR-Cas9 system, which is most widely used for genome editing tools, sometimes induces off-target, mosaic mutations, and small indels which cause unexpected phenotypes. Recently, we have reported that genome editing using CRISPR-Cas3 system is possible in human cells. This novel genomic editing system rarely induces off-target and mosaic mutations. We aim for the development of a safe and effective therapy for human cancers with the use of the CRISPR-Cas3 system.

We focus on chimeric antigen receptor T (CAR-T) cell therapy, which is an effective cancer immunotherapy. However, the standard strategy for producing CAR-T cells is expensive due to using autologous T cells. In order to generate autologous CAR-T cells, we investigated whether CRISPR-Cas3 system induces genetic modifications on genes involved in graft-versus-host disease and immune rejection in Jurkat cells, a human acute T cell leukemia cell line. As a result of this experiment, it caused loss of function of the target genes in its cells. In addition, we showed that this system generate targeted deletions of the target genes in human primary T cells. This result indicates that CRISPR-Cas3 system could be a genetic tool for generating allogenic CAR-T cells.

Photoactivatable Cre knock-in mice and rats for spatiotemporal control of genetic engineering in vivo

Kazuto Yoshimi, Yuko Yamauchi, Takao Tanaka[1], Toshio Shimada[1], Moritoshi Sato[2], Miho Hoshi, Saeko Ishida, Tomoji Mashimo
1, KAC Co. Ltd., Kyoto, 604-8423, Japan
2, Graduate School of Arts and Sciences, The University of Tokyo, Tokyo 153-8902, Japan

Although the Cre-loxP recombination system has been extensively used to analyze gene function in vivo, spatiotemporal control of Cre activity is a critical limitation for easy and precise recombination. We have previously established photoactivatable-Cre (PA-Cre) knock-in mice at safe harbor locus for the spatial and temporal regulation of Cre recombinase activity. The mice showed Cre recombination activity in a whole-body following light exposure for only 1 h. Almost no leaks of Cre recombination activity were detected in the knock-in mouse under natural light conditions. Spot irradiation could induce locus-specific recombination noninvasively, enabling us to compare phenotypes on the left and right sides in the same mouse. Furthermore, long-term irradiation using an implanted wireless LED substantially improved Cre recombination activity, especially in the brain. Furthermore, we recently developed PA-Cre KI rats and are characterizing their phenotypes. These results demonstrates that the PA-Cre knock-in mice and rats can facilitate spatiotemporal control of genetic engineering and promise a useful resource to elucidate gene function in vivo with Cre-loxP.

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Laboratory Animal Research Center

Animal Center
動物センター

Professor	Tomoji Mashimo, Ph.D.	教　授	博士(人間・環境学)	真　下　知　士
Senior Assistant Professor	Kazuto Yoshimi, Ph.D.	講　師	博士(医科学)	吉　見　一　人
Assistant Professor	Saeko Ishida, D.V.M., Ph.D.	助　教	博士(医学)	石　田　紗恵子

The Laboratory Animal Research Center (LARC) was founded in 1965 as the first modern animal facility in Japan. Currently about 21,090 mice are housed for research of IMSUT, and strictly maintained in the SPF condition. The Animal Center building of LARC was improved in 1998 to perform genome engineering in animals, to make infectious experiments (P2A, P3A), and to house bigger animals, such as rats and rabbits. Techniques of mouse embryo manipulation and generating genetically modified mice, including genome editing technologies, have been introduced into the LARC.

Animal Husbandry and Housing

The Animal Center building is a centralized facility designed, constructed and maintained to meet regulatory standards required for the operation of research animal facilities. We provide barrier housing rooms to support the production and use of genetically-engineered mice, biohazardous experiments area and equipment room which has X-ray Irradiator, MRI, CT and IVIS imaging system. In 2021 447 researchers from 42 laboratories are engaged in this facility with about 21,090 mice.

Techniques of mouse embryo manipulation (Microbiological cleaning and cryopreservation)

Our Barrie housing rooms are strictly maintained in the SPF condition; therefore, we provide IVF mouse derivation service for all mice shipped to LARC from other institutions or non-approved vendors to keep mice in SPF grade. We make frozen sperm and embryo for reducing number of using animals and laboratory space. In addition, it is useful for making back up of the strains. In 2021, 30strains of embryos and 36 strains of sperm were stored, and 155 tubes of frozen embryo were used for rederivation.

Amami Laboratory of Injurious Animals
奄美病害動物研究施設

Professor	Tomoji Mashimo, Ph.D.	教　授　博士(人間・環境学)	真　下　知　士
Assistant Professor	Shin-Ichi Yokota, D.V.M., Ph.D.	助　教　博士(人間科学)	横　田　伸　一

The Amami Laboratory of Injurious Animals (since 1970) has a long history originating from the branch office of the Institute for Infectious Disease which was established in 1902. We have made great achievements in filariasis eradication from this island and prevention of Habu bites. Currently, we are maintaining the colonies of New World monkeys, and aiming to overcome endemic infectious diseases in the tropical and subtropical regions through infection experiments using primates.

Reproduction of squirrel monkeys and owl monkeys

The squirrel monkey (*Saimiri boliviensis*) and the owl monkey (*Aotus lemurinus griseimenbra*) are widely distributed in the tropical rainforest in Central and South America. The advantage of using both species for medical researches resides in its small size and gentle behavior. Squirrel monkeys and owl monkeys are phylogenetically close to each other, and both are very famous as the best of malaria model in primates. In this laboratory, squirrel monkeys have a breeding season between winter and early spring. They are polygamy. Their puberty is 3-4 years old in females and 4-5 years old in males. Their gestation period is about 150 days. In contrast, owl monkeys are annual breeding animals. They are monogamy. Their puberty is 3 years old for both sex. Their gestation period is about 130 days. Ten newborns were given in reproductive groups of squirrel monkeys in 2021, and five of them were nursed by laboratory staffs due to neglect from their mothers. On the other hand, owl monkeys have become male-only colonies, and breeding has stopped at present.

Research using non-human primates

Our laboratory is the unique International Joint Usage and Research Center capable of conducting infection experiment using squirrel monkeys, owl monkeys, and cynomolgus monkeys. Our laboratory has now been closed BSL3 animal experimental rooms, but finished renovating animal experimental rooms up to BSL2 for infection experiments in primates in early 2021. We are working with collaborators from several institutions to develop an experimental squirrel monkey infection model to assess the anti-malarial activity of new compounds and vaccines. In 2021, we focused on in vitro experiments which we were able to conduct under COVID19 epidemic, and succeeded in establishing the staining method for cell surface markers of peripheral blood mononuclear cells in squirrel monkey.

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Medical Proteomics Laboratory
疾患プロテオミクスラボラトリー

Professor	Mutsuhiro Takekawa, M.D., Ph.D.	教　授　博士(医学)	武　川　睦　寛
Professor	Kouhei Tsumoto, Ph.D.	教　授　博士(工学)	津　本　浩　平
Associate Professor	Masaaki Oyama, Ph.D.	准教授　博士(医学)	尾　山　大　明
Senior Assistant Professor	Daisuke Kuroda, Ph.D.	講　師　博士(理学) (大学院工学系研究科)	黒　田　大　祐
Senior Assistant Professor	Makoto Nakakido, Ph.D.	講　師　博士(生命科学) (大学院工学系研究科)	中木戸　　誠
Assistant Professor	Ryo Matsunaga, Ph.D.	助　教　博士(生命科学) (大学院工学系研究科)	松　長　　遼
Project Assistant Professor	Hiroshi Sagara, Ph.D.	特任助教　博士(医学)	相　良　　洋

The mission of our laboratory is to develop advanced technologies for integrative proteomic analyses from a physicochemical, structural and systems biology point of view. Currently, we mainly focus on functional protein-protein interaction networks related to a variety of diseases including cancer and infection. We are also engaged in collaborative researches regarding mass spectrometry and electron microscopy, which have made a substantial contribution to many scientific achievements.

<Group I>

1. High-resolution proteomic analysis of EGF-regulated ubiquitination dynamics in human cancer cells

Hiroko Kozuka-Hata, Tomoko Hiroki, Aya Kitamura, Aiko Aizawa, Naoaki Miyamura, Kouhei Tsumoto, Jun-ichiro Inoue and Masaaki Oyama.

Protein ubiquitination is one of the most prevalent post-translational modifications (PTMs) and plays critical roles in regulating protein degradation, signal transduction and DNA repair in cooperation with other PTMs such as phosphorylation and acetylation. Recent mass spectrometry-based proteomics coupled with efficient enrichment technologies for each type of the modified peptides has enabled us to identify precise modification sites and measure their quantitative changes on a global scale. Our previous lysine-modification proteomic analyses of thirteen representative human cancer cell lines led us to identify thousands of ubiquitination (Ub) and acetylation (Ac) sites in total and revealed that their system-wide modification status was mutually different at the cellular network level. In this study, we further applied SILAC (Stable Isotope Labeling by Amino acids in Cell culture) for quantitative description of EGF-dependent lysine-modification site dynamics in HeLa cells in a time-resolved manner. Through integration of large-scale SILAC-encoded data on six time points upon EGF stimulation, we successfully quantified approximately 1,000 kinds of Ub-sites as well as 700 kinds of Ac-sites and found that one-third of these Ub-modified molecules, including several EGF signaling effectors, were subjected to downregulation by proteasomal inhibition.

2. Proteome-wide analysis of lysine acetylation and ubiquitination reveals critical signaling regulation in cancer cells

Hiroko Kozuka-Hata, Aya Kitamura, Tomoko Hiroki, Aiko Aizawa, Kouhei Tsumoto, Jun-ichiro Inoue and Masaaki Oyama.

Post-translational modifications (PTMs), such as

phosphorylation, ubiquitination and acetylation, are known to be widely involved in the regulation of various biological processes through extensive diversification of each protein function at the cellular network level. Previous functional analyses of cancer cell signaling under a variety of experimental conditions revealed many of the key molecules and their associated protein modifications in relation to each type of cancer. In order to systematically discover critical modulators from diversified signaling molecules, we have developed a high-resolution mass spectrometry-based proteomics platform for integrative identification and quantification of multiple post-translational modifications from various types of cancer cells. Our large-scale proteomic analysis enabled us to identify more than 5,000 kinds of ubiquitinated sites and 1,600 kinds of acetylated sites from representative human cancer cell lines, leading to identification of approximately 900 novel lysine modification sites in total. Very interestingly, 236 lysine residues derived from 141 proteins were found to be modified with both ubiquitination and acetylation. As a consequence of the subsequent motif extraction analyses, glutamic acid (E) was found to be highly enriched at the position (-1) for the lysine acetylation sites, whereas the same amino acid was relatively dispersed along the neighboring residues of the lysine ubiquitination sites.

3. System-wide perturbation of the proteome and phosphoproteome dynamics in glioblastoma stem cells through mTOR signaling inhibition

Hiroko Kozuka-Hata, Tomoko Hiroki, Ryo Koyama-Nasu[1], Kouhei Tsumoto, Jun-ichiro Inoue, Tetsu Akiyama[1] and Masaaki Oyama; [1]Laboratory of Molecular and Genetic Information, Institute for Quantitative Biosciences, The University of Tokyo

As glioblastoma is the most common and aggressive brain tumor with poor prognosis, systematic elucidation of signaling networks causally linked to the tumorigenesis is very crucial for developing more effective treatments for this intractable cancer. In our previous study, we applied a high-resolution mass spectrometry-based proteomics technology in combination with SILAC quantitative methods to understand EGF-dependent phosphoproteome dynamics in patient-derived glioblastoma stem cells. We demonstrated that the phosphorylation levels of the representative mTOR signaling molecules such as RPS6 and PRAS40 were dramatically up-regulated upon EGF stimulation. As EGFR signaling has been reported to play a pivotal role in regulating the maintenance of cancer stem cells, we next carried out mTOR inhibitor-dependent signaling perturbations to unravel stemness-related pathways at the network level.

In the present study, we identified a total of 3,726 proteins including 49 up-regulated and 436 down-regulated factors by Torin 1 treatment. Interestingly, we found that one of the well-known cancer stem cell markers was significantly down-regulated through mTOR signaling inhibition. Our in-depth phosphoproteome analysis also led to identification of 6,250 unique phosphopeptides derived from 2,221 proteins and unveiled a variety of dynamic changes regarding phosphorylation levels of cancer and neural stem cell markers in a comprehensive manner. The integrative view of the mTOR inhibitor-dependent proteome and phosphoproteome dynamics in glioblastoma stem cells presents us with further prospects towards understanding previously unrecognized regulations at the system level.

4. System-level analysis of CagA-dependent signaling network dynamics by Helicobacter pylori infection

Hiroko Kozuka-Hata, Masato Suzuki[2], Kotaro Kiga[2], Shinya Tasaki, Jun-ichiro Inoue, Tadashi Yamamoto[3], Chihiro Sasakawa[2] and Masaaki Oyama: [2]Division of Bacterial Infection, Department of Microbiology and Immunology, IMSUT, [3]Division of Oncology, Department of Cancer Biology, IMSUT

The signal transduction system within a cell regulates complex biological events in response to bacterial infection. The previous analyses of cell signaling in Helicobacter pylori-infected gastric epithelial cells have revealed that CagA, a major virulence factor of Helicobacter pylori, is delivered into cells via the type IV secretion system and perturbs signaling networks through the interaction with the key signaling molecules such as SHP-2, Grb2, Crk/Crk-L, Csk, Met, and ZO-1. Although the biological activity of tyrosine-phosphorylated CagA has intensively been studied, system-wide effects of the virulence factor on cellular signaling have yet to be analyzed. Here we performed time-resolved analyses of phosphoproteome and CagA-interactome in human gastric AGS cells by CagA-positive/negative Helicobacter pylori infection. Our highly sensitive nanoLC-MS/MS analyses in combination with the Stable Isotope Labeling by Amino acids in Cell culture (SILAC) technology defined CagA-dependent perturbation of signaling dynamics along with a subset of CagA-associated possible modulators on a network-wide scale. Our result indicated that the activation level of the phosphotyrosine-related signaling molecules in AGS cells was suppressed overall in the presence of CagA during Helicobacter pylori infection. As Helicobacter pylori infection plays pivotal roles in the progression of gastric diseases including carcinogenesis, a comprehensive and fine description of the signaling dynamics would serve as a fundamental platform to theoretically explore for the potential drug targets through ana-

lyzing the regulatory mechanisms at the system-level.

5. Mass spectrometry-based annotation of the human short ORFeome

Masaaki Oyama, Hiroko Kozuka-Hata, Sumio Sugano[4], Tadashi Yamamoto[3] and Jun-ichiro Inoue: [4]Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo

In parallel with the human genome projects, human full-length cDNA data has also been intensively accumulated. Large-scale analysis of their 5'-UTRs revealed that about half of these had a short ORF upstream of the coding region. Experimental verification as to whether such upstream ORFs are translated is essential to reconsider the generality of the classical scanning mechanism for initiation of translation and define the real outline of the human proteome. Our previous proteomics analysis of small proteins expressed in human K562 cells provided the first direct evidence of translation of upstream ORFs in human full-length cDNAs (Oyama et al., Genome Res, 14: 2048-2052, 2004). In order to grasp an expanded landscape of the human short ORFeome, we have performed an in-depth proteomics analysis of human K562 and HEK293 cells using a two-dimensional nanoLC-MS/MS system. The results led to the identification of eight protein-coding regions besides 197 small proteins with a theoretical mass less than 20 kDa that were already annotated coding sequences in the curated mRNA database. In addition to the upstream ORFs in the presumed 5'-untranslated regions of mRNAs, bioinformatics analysis based on accumulated 5'-end cDNA sequence data provided evidence of novel short coding regions that were likely to be translated from the upstream non-AUG start site or from the new short transcript variants generated by utilization of downstream alternative promoters. Protein expression analysis of the *GRINL1A* gene revealed that translation from the most upstream start site occurred on the minor alternative splicing transcript, whereas this initiation site was not utilized on the major mRNA, resulting in translation of the downstream ORF from the second initiation codon. These findings reveal a novel post-transcriptional system that can augment the human proteome via the alternative use of diverse translation start sites coupled with transcriptional regulation through alternative promoters or splicing, leading to increased complexity of short protein-coding regions defined by the human transcriptome (Oyama et al., Mol Cell Proteomics, 6: 1000-1006, 2007).

6. Shotgun proteomics deciphered age/division of labor-related functional specification of three honeybee (*Apis mellifera* L.) exocrine glands

Toshiyuki Fujita[5], Hiroko Kozuka-Hata, Yutaro Hori[5], Jun Takeuchi[5], Takeo Kubo[5], and Masaaki Oyama: [5]Department of Biological Sciences, Graduate School of Science, The University of Tokyo

The honeybee (*Apis mellifera* L.) uses various chemical signals produced by the worker exocrine glands to maintain the functioning of its colony. The roles of worker postcerebral glands (PcGs), thoracic glands (TGs), and mandibular glands (MGs) and the functional changes they undergo according to the division of labor from nursing to foraging are not as well studied. To comprehensively characterize the molecular roles of these glands in workers and their changes according to the division of labor of workers, we analyzed the proteomes of PcGs, TGs, and MGs from nurse bees and foragers using shotgun proteomics technology. We identified approximately 2000 proteins from each of the nurse bee or forager glands and highlighted the features of these glands at the molecular level by semiquantitative enrichment analyses of frequently detected, gland-selective, and labor-selective proteins. First, we found the high potential to produce lipids in PcGs and MGs, suggesting their relation to pheromone production. Second, we also found the proton pumps abundant in TGs and propose some transporters possibly related to the saliva production. Finally, our data unveiled candidate enzymes involved in labor-dependent acid production in MGs.

7. In-depth proteomic analysis of drug-responsive signaling pathway elements in human cancer cells

Wei QI, Aya Kitamura, Naoaki Miyamura, Tomoko Hiroki, Aiko Aizawa, Kazuki Mori, Hiroko Kozuka-Hata and Masaaki Oyama.

Abnormal expression of histone deacetylases (HDACs) in human cancer cells was reported to be associated with angiogenesis, migration, chemotherapy resistance as well as cell differentiation and apoptosis in a wide range of previous studies. Therefore, clinical use of HDAC inhibitors has been discussed as a new therapeutic approach against cancer for a long period. In 2006, suberoylanilide hydroxamic acid (SAHA), a pan-inhibitor targeting HDACs and also known as Vorinostat, was approved by the US Food and Drug Administration (FDA) for the treatment of cutaneous T-cell lymphoma. In addition to the anticancer activity against hematologic cancers, SAHA also shows a significant antitumor effect on solid tumors through inducing apoptosis, arresting cell cycle

or elevating radiation sensitization. In order to unveil the underlying complex mechanism, we used human HeLa cells as the model platform for analyzing SAHA-responsive elements on a proteomic scale. According to the experimental pre-evaluation through western blotting for acetylated histone H3 and microscopic observation of cell growth under a variety of drug-perturbed conditions, we determined to treat cultured cells with SAHA for 24 h to perform an in-depth quantitative proteomic analysis of SAHA-responsive elements in human HeLa cells. After SAHA treatment, the cells were lysed, trypsin-digested and analyzed by high-resolution nanoflow liquid chromatography- tandem mass spectrometry. As a result of ultra-deep proteomic analysis by Orbitrap Eclipse Tribrid system coupled with Ultimate 3000 RSLCnano liquid chromatography technology, a total of 5,135 proteins was identified using Proteome Discoverer software. Approximately 8 % of the identified proteins were found to be differentially regulated with more than two-fold changes in response to SAHA treatment by Label Free Quantification (LFQ). The subsequent pathway analysis based on Kyoto Encyclopedia of Genes and Genomes (KEGG) indicated that cell cycle and anti-apoptotic pathway elements including p27 and HO-1 were prominently correlated with SAHA-dependent regulation in human HeLa cells.

<Group II>

Biomolecular recognition is based on collective and specific non-covalent interactions between discrete biological molecules. Our laboratory studies various protein systems, for instance, antibody-antigen and protein-ligand complexes, to understand quantitatively how these coordinated non-covalent interactions contribute to their specific recognition in biological and artificial systems. We seek to elucidate the molecular mechanisms by which biological molecules obtain high-specificity and affinity from multiple angles using advanced instrumentation. To produce functional molecules with higher performance and better properties, we aim to build a solid foundation from which to develop drugs that modulate specific interactions between biomolecules and ultimately to understand the principles of molecular interactions in our lives.

1. Structural basis for antigen recognition by methylated lysine-specific antibodies

Ishii M, Nakakido M, Caaveiro JMM, Kuroda D, Okumura CJ, Maruyama T, Entzminger K and Tsumoto K.

Proteins are modulated by a variety of posttranslational modifications including methylation. Despite its importance, the majority of protein methylation modifications discovered by mass spectrometric analyses are functionally uncharacterized, partly owing to the difficulty in obtaining reliable methylsite-specific antibodies. To elucidate how functional methylsite-specific antibodies recognize the antigens and lead to the development of a novel method to create such antibodies, we use an immunized library paired with phage display to create rabbit monoclonal antibodies recognizing trimethylated Lys260 of MAP3K2 as a representative substrate. We isolated several methylsite-specific antibodies that contained unique complementarity determining region sequence. We characterized the mode of antigen recognition by each of these antibodies using structural and biophysical analyses, revealing the molecular details, such as binding affinity toward methylated/nonmethylated antigens and structural motif that is responsible for recognition of the methylated lysine residue, by which each antibody recognized the target antigen. In addition, the comparison with the results of Western blotting analysis suggests a critical antigen recognition mode to generate cross-reactivity to protein and peptide antigen of the antibodies. Computational simulations effectively recapitulated our biophysical data, capturing the antibodies of differing affinity and specificity. Our exhaustive characterization provides molecular architectures of functional methylsite-specific antibodies and thus should contribute to the development of a general method to generate functional methylsite-specific antibodies by de novo design.

2. Proteomic identification and validation of novel interactions of the putative tumor suppressor PRELP with membrane proteins including IGFI-R and p75NTR

Kosuge H, Nakakido M, Nagatoishi S, Fukuda T, Bando Y, Ohnuma SI and Tsumoto K.

Proline and arginine-rich end leucine-rich repeat protein (PRELP) is a member of the small leucine-rich repeat proteoglycans (SLRPs) family. Levels of PRELP mRNA are downregulated in many types of cancer, and PRELP has been reported to have suppressive effects on tumor cell growth, although the molecular mechanism has yet to be fully elucidated. Given that other SLRPs regulate signaling pathways through interactions with various membrane proteins, we reasoned that PRELP likely interacts with membrane proteins to maintain cellular homeostasis. To identify membrane proteins that interact with PRELP, we carried out coimmunoprecipitation coupled with mass spectrometry (CoIP-MS). We prepared membrane fractions from Expi293 cells transfected to overexpress FLAG-tagged PRELP or control cells and analyzed samples precipitated with anti-FLAG antibody by mass spectrometry. Comparison of membrane proteins in each sample identified several that seem to interact with PRELP; among them, we noted two growth factor receptors, insulin-like growth factor I

receptor (IGFI-R) and low-affinity nerve growth factor receptor (p75NTR), interactions with which might help to explain PRELP's links to cancer. We demonstrated that PRELP directly binds to extracellular domains of these two growth factor receptors with low micromolar affinities by surface plasmon resonance analysis using recombinant proteins. Furthermore, cell-based analysis using recombinant PRELP protein showed that PRELP suppressed cell growth and affected cell morphology of A549 lung carcinoma cells, also at micromolar concentration. These results suggest that PRELP regulates cellular functions through interactions with IGFI-R and p75NTR and provide a broader set of candidate partners for further exploration

3. Structural basis for selective inhibition of human serine hydroxymethyltransferase by secondary bile acid conjugate

Ota T, Senoo A, Shirakawa M, Nonaka H, Saito Y, Ito S, Ueno G, Nagatoishi S, Tsumoto K and Sando S.

Bile acids are metabolites of cholesterol that facilitate lipid digestion and absorption in the small bowel. Bile acids work as agonists of receptors to regulate their own metabolism. Bile acids also regulate other biological systems such as sugar metabolism, intestinal multidrug resistance, and adaptive immunity. However, numerous physiological roles of bile acids remain undetermined. In this study, we solved the crystal structure of human serine hydroxymethyltransferase (hSHMT) in complex with an endogenous secondary bile acid glycine conjugate. The specific interaction between hSHMT and the ligand was demonstrated using mutational analyses, biophysical measurements, and structure-activity relationship studies, suggesting that secondary bile acid conjugates may act as modulators of SHMT activity.

4. Molecular basis for the activation of actinoporins by lipids

Caaveiro JMM and Tsumoto K.

Actinoporins are a family of homologous pore forming proteins from sea anemones. They are one of the few families of eukaryotic toxins that have been characterized in depth. Actinoporins are activated by lipids in the context of bilayers, especially in cell and in model membranes containing the lipid sphingomyelin. These proteins must undergo conformational changes induced upon interaction with lipids in the membrane, where they form cytotoxic pores causing cell death and lethality. Herein we review a list of procedures and techniques to study this family of toxins, with the goal of elucidating the physicochemical, thermodynamic and structural basis for their activation by lipids. The emerging picture indicates that actinoporins undergo a stepwise process that includes binding to the membrane, oligomerization, and pore formation, in this order. The key transformation from the inactive oligomer to the active pore is catalyzed by sphingomyelin, explaining the key role of this lipid in the function of actinoporins.

5. A new twist of rubredoxin function in M. tuberculosis

Sushko T, Kavaleuski A, Grabovec I, Kavaleuskaya A, Vakhrameev D, Bukhdruker S, Marin E, Kuzikov A, Masamrekh R, Shumyantseva V, Tsumoto K, Borshchevskiy V, Gilep A and Strushkevich N.

Electron transfer mediated by metalloproteins drives many biological processes. Rubredoxins are a ubiquitous [1Fe-0S] class of electron carriers that play an important role in bacterial adaptation to changing environmental conditions. In Mycobacterium tuberculosis, oxidative and acidic stresses as well as iron starvation induce rubredoxins expression. However, their functions during M. tuberculosis infection are unknown. In the present work, we show that rubredoxin B (RubB) is able to efficiently shuttle electrons from cognate reductases, FprA and FdR to support catalytic activity of cytochrome P450s, CYP124, CYP125, and CYP142, which are important for bacterial viability and pathogenicity. We solved the crystal structure of RubB and characterized the interaction between RubB and CYPs using site-directed mutagenesis. Mutations that not only neutralize single charge but also change the specific residues on the surface of RubB did not dramatically decrease activity of studied CYPs. Together with isothermal calorimetry (ITC) experiments, the obtained results suggest that interactions are transient and not highly specific. The redox potential of RubB is -264 mV vs. Ag/AgCl and the measured extinction coefficients are 9931 M-1cm-1 and 8371 M-1cm-1 at 380 nm and 490 nm, respectively. Characteristic parameters of RubB along with the discovered function might be useful for biotechnological applications. Our findings suggest that a switch from ferredoxins to rubredoxins might be crucial for M. tuberculosis to support CYPs activity during the infection.

6. Heme controls the structural rearrangement of its sensor protein mediating the hemolytic bacterial survival

Nishinaga M, Sugimoto H, Nishitani Y, Nagai S, Nagatoishi S, Muraki N, Tosha T, Tsumoto K, Aono S, Shiro Y and Sawai H.

Hemes (iron-porphyrins) are critical for biological processes in all organisms. Hemolytic bacteria survive by acquiring b-type heme from hemoglobin in

red blood cells from their animal hosts. These bacteria avoid the cytotoxicity of excess heme during hemolysis by expressing heme-responsive sensor proteins that act as transcriptional factors to regulate the heme efflux system in response to the cellular heme concentration. Here, the underlying regulatory mechanisms were investigated using crystallographic, spectroscopic, and biochemical studies to understand the structural basis of the heme-responsive sensor protein PefR from Streptococcus agalactiae, a causative agent of neonatal life-threatening infections. Structural comparison of heme-free PefR, its complex with a target DNA, and heme-bound PefR revealed that unique heme coordination controls a >20 Å structural rearrangement of the DNA binding domains to dissociate PefR from the target DNA. We also found heme-bound PefR stably binds exogenous ligands, including carbon monoxide, a by-product of the heme degradation reaction.

7. A DNA Aptamer That Inhibits the Aberrant Signaling of Fibroblast Growth Factor Receptor in Cancer Cells

Eguchi A, Ueki A, Hoshiyama J, Kuwata K, Chikaoka Y, Kawamura T, Nagatoishi S, Tsumoto K, Ueki R and Sando S.

Growth factor receptors are activated through dimerization by the binding of their ligands and play pivotal roles in normal cell function. However, the aberrant activity of the receptors has been associated with cancer malignancy. One of the main causes of the aberrant receptor activation is the overexpression of receptors and the resultant formation of unliganded receptor dimers, which can be activated in the absence of external ligand molecules. Thus, the unliganded receptor dimer is a promising target to inhibit aberrant signaling in cancer. Here, we report an aptamer that specifically binds to fibroblast growth factor receptor 2b and inhibits the aberrant receptor activation and signaling. Our investigation suggests that this aptamer inhibits the formation of the receptor dimer occurring in the absence of external ligand molecules. This work presents a new inhibitory function of aptamers and the possibility of oligonucleotide-based therapeutics for cancer.

8. Anti-EGFR antibody 528 binds to domain III of EGFR at a site shifted from the cetuximab epitope

Makabe K, Yokoyama T, Uehara S, Uchikubo-Kamo T, Shirouzu M, Kimura K, Tsumoto K, Asano R, Tanaka Y and Kumagai I.

Antibodies have been widely used for cancer therapy owing to their ability to distinguish cancer cells by recognizing cancer-specific antigens. Epidermal growth factor receptor (EGFR) is a promising target for the cancer therapeutics, against which several antibody clones have been developed and brought into therapeutic use. Another antibody clone, 528, is an antagonistic anti-EGFR antibody, which has been the focus of our antibody engineering studies to develop cancer drugs. In this study, we explored the interaction of 528 with the extracellular region of EGFR (sEGFR) via binding analyses and structural studies. Dot blotting experiments with heat treated sEGFR and surface plasmon resonance binding experiments revealed that 528 recognizes the tertiary structure of sEGFR and exhibits competitive binding to sEGFR with EGF and cetuximab. Single particle analysis of the sEGFR-528 Fab complex via electron microscopy clearly showed the binding of 528 to domain III of sEGFR, the domain to which EGF and cetuximab bind, explaining its antagonistic activity. Comparison between the two-dimensional class average and the cetuximab/sEGFR crystal structure revealed that 528 binds to a site that is shifted from, rather than identical to, the cetuximab epitope, and may exclude known drug-resistant EGFR mutations.

9. Thermodynamic Dissection of Potency and Selectivity of Cytosolic Hsp90 Inhibitors.

Yoshimura C, Nagatoishi S, Kuroda D, Kodama Y, Uno T, Kitade M, Chong-Takata K, Oshiumi H, Muraoka H, Yamashita S, Kawai Y, Ohkubo S and Tsumoto K.

The cytosolic Hsp90-selective inhibitor TAS-116 has an acceptable safety profile and promising antitumor activity in clinical trials. We examined the binding characteristics of TAS-116 and its analogs to determine the impact of the ligand binding mode on selectivity for cytosolic Hsp90. Analyses of the co-crystal structure of Hsp90 and inhibitor TAS-116 suggest that TAS-116 interacts with the ATP-binding pocket, the ATP lid region, and the hydrophobic pocket. A competitive isothermal titration calorimetry analysis confirmed that a small fragment of TAS-116 (THS-510) docks into the lid region and hydrophobic pockets without binding to the ATP-binding pocket. THS-510 exhibited enthalpy-driven binding to Hsp90α and selectively inhibited cytosolic Hsp90 activity. The heat capacity change of THS-510 binding was positive, likely due to the induced conformational rearrangement of Hsp90. Thus, we concluded that interactions with the hydrophobic pocket of Hsp90 determine potency and selectivity of TAS-116 and derivatives for the cytosolic Hsp90 isoform

10. A Novel Cell-Based Intracellular Protein-Protein Interaction Detection Platform (SOLIS) for Multimodality Screening

Kashima D, Kageoka M, Kimura Y, Horikawa M,

Miura M, Nakakido M, Tsumoto K, Nagamune T and Kawahara M.

Intervention in protein-protein interactions (PPIs) has tremendous effects in the molecular therapy of many diseases. To fulfill the requirements for targeting intracellular proteins, here we develop SOS-localization-based interaction screening (SOLIS), which elaborately mimics signaling via the Ras-mitogen-activated protein kinase pathway. SOLIS employs two chimeric proteins in which a membrane localization motif (CaaX) is fused at the C-terminus of a protein of interest and the catalytic domain of SOS is fused at the C-terminus of another protein of interest. Interaction between the two proteins of interest induces membrane localization of the SOS chimera and cell proliferation. Thus, the SOLIS system enables enrichment of superior binders based on cell proliferation in an intracellular PPI-dependent manner. This was verified by three major modalities against intracellular PPIs (small molecules, peptide aptamers, and intrabodies). The system worked over a broad range of affinities (KD = 0.32-140 nM). In a screening of a site-directed randomized library, novel intrabody clones were selected on the basis of the potency of cell proliferation. Three other PPI detection methods (NanoBiT, SPR, and pull-down assays) were employed to characterize the SOLIS system, and several intrabody clones were judged as false negatives in these assays. SOLIS signals would be less sensitive to the orientation/conformation of the chimeric proteins, and this feature emerges as the advantage of SOLIS as a mammalian cytosolic PPI detection system with few false negatives.

11. Novel neutralizing human monoclonal antibodies against tetanus neurotoxin

Minamitani T, Kiyose K, Otsubo R, Ito T, Akiba H, Furuta RA, Inoue T, Tsumoto K, Satake M and Yasui T.

Tetanus is a fatal disease caused by tetanus neurotoxin (TeNT). TeNT is composed of a light chain (Lc) and a heavy chain, the latter of which is classified into two domains, N-terminus Hn and C-terminus Hc. Several TeNT-neutralizing antibodies have been reported, but it remains unclear which TeNT domains are involved in neutralization. To further understand the mechanism of these antibodies, we isolated TeNT-reactive human antibody clones from peripheral blood mononuclear cells. We then analyzed the reactivity of the isolated antibody clones to each protein domain and their inhibition of Hc-ganglioside GT1b binding, which is critical for TeNT toxicity. We also investigated the TeNT-neutralizing ability of isolated antibody clones and showed that an Hn-reactive clone protected strongly against TeNT toxicity in mice. Furthermore, combination treatment of Hn-reactive antibody clones with both Hc-reactive and TeNT mix (the mixture of Hc, Hn, and Lc proteins)-reactive antibody clones enhanced the neutralizing effect. These results indicated that antibody clones targeting Hn effectively neutralized TeNT. In addition, the use of a cocktail composed of Hc-, Hn-, and TeNT mix-reactive antibodies provided enhanced protection compared to the use of each antibody alone.

12. Elaboration of Non-naturally Occurring Helical Tripeptides as p53-MDM2/MDMX Interaction Inhibitors

Su A, Tabata Y, Aoki K, Sada A, Ohki R, Nagatoishi S, Tsumoto K, Wang S, Otani Y and Ohwada T.

Protein-protein interactions (PPIs) are often mediated by helical, strand and/or coil secondary structures at the interface regions. We previously showed that non-naturally occurring, stable helical trimers of bicyclic β-amino acids (Abh) with all-trans amide bonds can block the p53-MDM2/MDMX α-helix-helix interaction, which plays a role in regulating p53 function. Here, we conducted docking and molecular dynamics calculations to guide the structural optimization of our reported compounds, focusing on modifications of the C-terminal/N-terminal residues. We confirmed that the modified peptides directly bind to MDM2 by means of thermal shift assay, isothermal titration calorimetry, and enzyme-linked immunosorbent assay (ELISA) experiments. Biological activity assay in human osteosarcoma cell line SJSA-1, which has wild-type p53 and amplification of the Mdm2 gene, indicated that these peptides are membrane-permeable p53-MDM2/MDMX interaction antagonists that can rescue p53 function in the cells.

13. Anion solvation enhanced by positive supercharging mutations preserves thermal stability of an antibody in a wide pH range

Kasahara K, Kuroda D, Tanabe A, Kawade R, Nagatoishi S and Tsumoto K.

Proteins function through interactions with other molecules. In protein engineering, scientists often engineer proteins by mutating their amino acid sequences on the protein surface to improve various physicochemical properties. “Supercharging” is a method to design proteins by mutating surface residues with charged amino acids. Previous studies demonstrated that supercharging mutations conferred better thermal resistance, solubility, and cell penetration to proteins. Likewise, antibodies recognize antigens through the antigen-binding site on the surface. The genetic and structural diversity of antibodies leads to high specificity and affinity toward antigens, enabling antibodies to be versatile tools in various applications. When assessing therapeutic an-

tibodies, surface charge is an important factor to consider because the isoelectric point plays a role in protein clearance inside the body. In this study, we explored how supercharging mutations affect physicochemical properties of antibodies. Starting from a crystal structure of an antibody with the net charge of -4, we computationally designed a supercharged variant possessing the net charge of +10. The positive-supercharged antibody exhibited marginal improvement in thermal stability, but the secondary structure and the binding affinity to the antigen (net charge of +8) were preserved. We also used physicochemical measurements and molecular dynamics simulations to analyze the effects of supercharging mutations in sodium phosphate buffer with different pH and ion concentrations, which revealed preferential solvation of phosphate ions to the supercharged surface relative to the wild-type surface. These results suggest that supercharging would be a useful approach to preserving thermal stability of antibodies in a wide range of pH, which may enable further diversification of antibody repertoires beyond natural evolution.

14. Peptoid-based reprogrammable template for cell-permeable inhibitors of protein-protein interactions

Fukuda Y, Yokomine M, Kuroda D, Tsumoto K, Morimoto J and Sando S.

The development of inhibitors of intracellular protein-protein interactions (PPIs) is of great significance for drug discovery, but the generation of a cell-permeable molecule with high affinity to protein is challenging. Oligo(N-substituted glycines) (oligo-NSGs), referred to as peptoids, are attractive as potential intracellular PPI inhibitors owing to their high membrane permeability. However, their intrinsically flexible backbones make the rational design of inhibitors difficult. Here, we propose a peptoid-based rational approach to develop cell-permeable PPI inhibitors using oligo(N-substituted alanines) (oligo-NSAs). The rigid structures of oligo-NSAs enable independent optimization of each N-substituent to improve binding affinity and membrane permeability, while preserving the backbone shape. A molecule with optimized N-substituents inhibited a target PPI in cells, which demonstrated the utility of oligo-NSA as a reprogrammable template to develop intracellular PPI inhibitors.

15. Single-chain variable fragment (scFv) targeting streptolysin O controls group A Streptococcus infection.

Aikawa C, Kawashima K, Fukuzaki C, Nakakido M, Murase K, Nozawa T, Tsumoto K and Nakagawa I.

Streptococcus pyogenes (Group A Streptococcus, GAS) causes a range of human diseases, including life-threatening and severe invasive GAS infections, such as streptococcal toxic shock syndrome (STSS). Several antibiotics, including penicillin, are effective against GAS. Still, invasive GAS diseases have a high mortality rate (>30%). Clinical isolates from STSS patients show higher expression of pore-forming streptolysin O (SLO). Thus, SLO is an important pathogenic factor for GAS and may be an effective target for treatment of GAS disease. We succeeded in obtaining a single-chain variable fragment (scFv) SLO-I4 capable of recognizing SLO, which significantly inhibited GAS-induced cell lytic activity in erythrocytes, macrophages, and epithelial cells. In epithelial cells, SLO-I4 significantly reduced SLO-mediated endosomal membrane damage, which consequently prevented bacterial escape from the endosome. The effectiveness of anti-SLO scFv in counteracting SLO function suggests that it might be beneficial against GAS infections.

16. Characterization of a putative maltodextrin-binding protein of Streptococcus pyogenes, SPs0871 and the development of a VHH inhibitor

Yamawaki T, Nakakido M, Ujiie K, Aikawa C, Nakagawa I and Tsumoto K.

Streptococcus pyogenes causes a wide range of human infections. Currently, antibiotics are the main treatment for S. pyogenes infection, but serious anti-microbial resistance requires alternative treatment options. To develop a novel strategy for treatment, we physicochemically characterized SPs0871, a putative maltose/maltodextrin-binding protein that is thought to have important roles in the pathogenesis of invasive streptococci. We obtained a variable domain of heavy chain of heavy-chain antibody, the smallest unit of an antibody, which specifically binds to SPs0871. Although the VHH completely inhibited the binding of maltodextrins to SPs0871, the inhibition did not lead to growth suppression of the bacteria. Our results provide important insights for development of VHH as an anti-streptococcal therapeutic.

17. Microsecond molecular dynamics suggest that a non-synonymous mutation, frequently observed in patients with mild symptoms in Tokyo, alters dynamics of the SARS-CoV-2 main protease

Kuroda D and Tsumoto K.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes the coronavirus disease 2019 (COVID-19), spread rapidly around the globe. The main protease encoded by SARS-CoV-2 is essen-

tial for processing of the polyproteins translated from the viral RNA genome, making this protein a potential drug target. A recently reported mutation in the protease, P108S, may be responsible for milder symptoms observed in COVID-19 patients in Tokyo. Starting from a crystal structure of the SARS-CoV-2 main protease in the dimeric form, we performed triplicate 5.0-μs molecular dynamics simulations of the wild-type and P108S mutant. Our computational results suggest a link between the mutation P108S and dynamics of the catalytic sites in the main protease: The catalytic dyad become considerably inaccessible to substrates in the P108S mutant. Our results also demonstrate the potential of molecular dynamics simulations to complement experimental techniques and other computational methods, such as protein design calculations, which predict effects of mutations based on static crystal structures. Further studies are certainly necessary to quantitively understand the relationships between the P108S mutation and physical properties of the main protease, but the results of our study will immediately inform development of new protease inhibitors.

18. A glutamine sensor that directly activates TORC1

Tanigawa M, Yamamoto K, Nagatoishi S, Nagata K, Noshiro D, Noda NN, Tsumoto K and Maeda T.

TOR complex 1 (TORC1) is an evolutionarily-conserved protein kinase that controls cell growth and metabolism in response to nutrients, particularly amino acids. In mammals, several amino acid sensors have been identified that converge on the multi-layered machinery regulating Rag GTPases to trigger TORC1 activation; however, these sensors are not conserved in many other organisms including yeast. Previously, we reported that glutamine activates yeast TORC1 via a Gtr (Rag ortholog)-independent mechanism involving the vacuolar protein Pib2, although the identity of the supposed glutamine sensor and the exact TORC1 activation mechanism remain unclear. In this study, we successfully reconstituted glutamine-responsive TORC1 activation in vitro using only purified Pib2 and TORC1. In addition, we found that glutamine specifically induced a change in the folding state of Pib2. These findings indicate that Pib2 is a glutamine sensor that directly activates TORC1, providing a new model for the metabolic control of cells.

19. Mechanism of dimerization and structural features of human LI-cadherin

Yui A, Caaveiro JMM, Kuroda D, Nakakido M, Nagatoishi S, Goda S, Maruno T, Uchiyama S and Tsumoto K

Liver intestine (LI)-cadherin is a member of the cadherin superfamily, which encompasses a group of Ca2 + -dependent cell-adhesion proteins. The expression of LI-cadherin is observed on various types of cells in the human body, such as normal small intestine and colon cells, and gastric cancer cells. Because its expression is not observed on normal gastric cells, LI-cadherin is a promising target for gastric cancer imaging. However, because the cell adhesion mechanism of LI-cadherin has remained unknown, rational design of therapeutic molecules targeting this cadherin has been hampered. Here, we have studied the homodimerization mechanism of LI-cadherin. We report the crystal structure of the LI-cadherin homodimer containing its first four extracellular cadherin repeats (EC1-4). The EC1-4 homodimer exhibited a unique architecture different from that of other cadherins reported so far, driven by the interactions between EC2 of one protein chain and EC4 of the second protein chain. The crystal structure also revealed that LI-cadherin possesses a noncanonical calcium ion-free linker between the EC2 and EC3 domains. Various biochemical techniques and molecular dynamics simulations were employed to elucidate the mechanism of homodimerization. We also showed that the formation of the homodimer observed in the crystal structure is necessary for LI-cadherin-dependent cell adhesion by performing cell aggregation assays. Taken together, our data provide structural insights necessary to advance the use of LI-cadherin as a target for imaging gastric cancer.

20. Regulation of cadherin dimerization by chemical fragments as a trigger to inhibit cell adhesion

Senoo A, Ito S, Nagatoishi S, Saito Y, Ueno G, Kuroda D, Yoshida K, Tashima T, Kudo S, Sando S and Tsumoto K.

Many cadherin family proteins are associated with diseases such as cancer. Since cell adhesion requires homodimerization of cadherin molecules, a small-molecule regulator of dimerization would have therapeutic potential. Herein, we describe identification of a P-cadherin-specific chemical fragment that inhibits P-cadherin-mediated cell adhesion. Although the identified molecule is a fragment compound, it binds to a cavity of P-cadherin that has not previously been targeted, indirectly prevents formation of hydrogen bonds necessary for formation of an intermediate called the X dimer and thus modulates the process of X dimerization. Our findings will impact on a strategy for regulation of protein-protein interactions and stepwise assembly of protein complexes using small molecules.

21. Production of IgG1-based bispecific antibody without extra cysteine residue via intein-mediated protein trans-splicing

Akiba H, Ise T, Nagata S, Kamada H, Ohno H and Tsumoto K.

A major class of bispecific antibodies (BsAbs) utilizes heterodimeric Fc to produce the native immunoglobulin G (IgG) structure. Because appropriate pairing of heavy and light chains is required, the design of BsAbs produced through recombination or reassembly of two separately-expressed antigen-binding fragments is advantageous. One such method uses intein-mediated protein trans-splicing (IMPTS) to produce an IgG1-based structure. An extra Cys residue is incorporated as a consensus sequence for IMPTS in successful examples, but this may lead to potential destabilization or disturbance of the assay system. In this study, we designed a BsAb linked by IMPTS, without the extra Cys residue. A BsAb binding to both TNFR2 and CD30 was successfully produced. Cleaved side product formation was inevitable, but it was minimized under the optimized conditions. The fine-tuned design is suitable for the production of IgG-like BsAb with high symmetry between the two antigen-binding fragments that is advantageous for screening BsAbs.

22. Delicate balance among thermal stability, binding affinity, and conformational space explored by single-domain V H H antibodies

Ikeuchi E, Kuroda D, Nakakido M, Murakami A and Tsumoto K.

The high binding affinities and specificities of antibodies have led to their use as drugs and biosensors. Single-domain VHH antibodies exhibit high specificity and affinity but have higher stability and solubility than conventional antibodies as they are single-domain proteins. In this work, based on physicochemical measurements and molecular dynamics (MD) simulations, we have gained insight that will facilitate rational design of single-chain VHH antibodies. We first assessed two homologous VHH antibodies by differential scanning calorimetry (DSC); one had a high (64.8 °C) and the other a low (58.6 °C) melting temperature. We then generated a series of the variants of the low stability antibody and analyzed their thermal stabilities by DSC and characterized their structures through MD simulations. We found that a single mutation that resulted in 8.2 °C improvement in melting temperature resulted in binding affinity an order of magnitude lower than the parent antibody, likely due to a shift of conformational space explored by the single-chain VHH antibody. These results suggest that the delicate balance among conformational stability, binding capability, and conformational space explored by antibodies must be considered in design of fully functional single-chain VHH antibodies.

23. Development of biparatopic bispecific antibody possessing tetravalent scFv-Fc capable of binding to ROBO1 expressed in hepatocellular carcinoma cells

Watanabe Y, Tanabe A, Hamakubo T, Nagatoishi S and Tsumoto K.

There is no standard structural format of the biparatopic bispecific antibody (bbsAb) which is used against the target molecule because of the diversity of biophysical features of bispecific antibodies (bsAbs). It is therefore essential that the interaction between the antibody and antigen is quantitatively analyzed to design antibodies that possess the desired properties. Here, we generated bsAbs, namely, a tandem scFv-Fc, a diabody-Fc, and an immunofusion-scFv-Fc-scFv, that possessed four scFv arms at different positions and were capable of recognizing the extracellular domains of ROBO1. We examined the interactions between these bsAbs and ROBO1 at the biophysical and cellular levels. Of these, immunofusion-B2212A scFv-Fc-B5209B scFv was stably expressed with the highest relative yield. The kinetic and thermodynamic features of the interactions of each bsAb with soluble ROBO1 (sROBO1) were validated using surface plasmon resonance and isothermal titration calorimetry. In all bsAbs, the immunofusion-scFv-Fc-scFv format showed homogeneous interaction with the antigen with higher affinity compared with that of monospecific antibodies. In conclusion, our study presents constructive information to design druggable bbsAbs in drug applications.

24. Electrostatic-triggered exothermic antibody adsorption to the cellulose nanoparticles

Murakami K, Nagatoishi S, Kasahara K, Nagai H, Sasajima Y, Sasaki R and Tsumoto K.

Antibody-conjugated nanoparticles are used in a fields ranging from medicine to engineering. NanoAct® nanobeads are cellulose nanoparticles used in lateral flow assays that are highly water dispersible. In order to promote the adsorption of antibodies onto NanoAct® particles while maintaining their activity, we analyzed the adsorption onto NanoAct® particles thermodynamically and elucidated the adsorption mechanism. In an immunochromatographic assay, the amount of adsorbed antibody and the color intensity of the test line increased as the pH decreased. The zeta potential of the nanoparticles remained constant at around -30 mV over the pH range from 2 to 10. The model antibody had pI values between 6.2 and 6.8. Isothermal calorimetry analysis showed that adsorp-

tion of antibody to the NanoAct® particle is an endothermic reaction under low pH conditions, an exothermic reaction between pH 6 and pH 7, and a weakly exothermic reaction above pH 7. These data indicate that the changes in net charge of the antibody surface as a function of pH influence the pH dependence of antibody adsorption to the negatively charged NanoAct®. This suggests that increased positive charge on the antibody surface will result in a more sensitive NanoAct®-based immunoassay.

25. An integrated computational pipeline for designing high-affinity nanobodies with expanded genetic codes

Padhi AK, Kumar A, Haruna KI, Sato H, Tamura H, Nagatoishi S, Tsumoto K, Yamaguchi A, Iraha F, Takahashi M, Sakamoto K and Zhang KYJ.

Protein engineering and design principles employing the 20 standard amino acids have been extensively used to achieve stable protein scaffolds and deliver their specific activities. Although this confers some advantages, it often restricts the sequence, chemical space, and ultimately the functional diversity of proteins. Moreover, although site-specific incorporation of non-natural amino acids (nnAAs) has been proven to be a valuable strategy in protein engineering and therapeutics development, its utility in the affinity-maturation of nanobodies is not fully explored. Besides, current experimental methods do not routinely employ nnAAs due to their enormous library size and infinite combinations. To address this, we have developed an integrated computational pipeline employing structure-based protein design methodologies, molecular dynamics simulations and free energy calculations, for the binding affinity prediction of an nnAA-incorporated nanobody toward its target and selection of potent binders. We show that by incorporating halogenated tyrosines, the affinity of 9G8 nanobody can be improved toward epidermal growth factor receptor (EGFR), a crucial cancer target. Surface plasmon resonance (SPR) assays showed that the binding of several 3-chloro-l-tyrosine (3MY)-incorporated nanobodies were improved up to 6-fold into a picomolar range, and the computationally estimated binding affinities shared a Pearson's r of 0.87 with SPR results. The improved affinity was found to be due to enhanced van der Waals interactions of key 3MY-proximate nanobody residues with EGFR, and an overall increase in the nanobody's structural stability. In conclusion, we show that our method can facilitate screening large libraries and predict potent site-specific nnAA-incorporated nanobody binders against crucial disease-targets.

26. The transcriptional corepressor CtBP2 serves as a metabolite sensor orchestrating hepatic glucose and lipid homeostasis

Ota T, Senoo A, Shirakawa M, Nonaka H, Saito Y, Ito S, Ueno G, Nagatoishi S, Tsumoto K and Sando S.

Biological systems to sense and respond to metabolic perturbations are critical for the maintenance of cellular homeostasis. Here we describe a hepatic system in this context orchestrated by the transcriptional corepressor C-terminal binding protein 2 (CtBP2) that harbors metabolite-sensing capabilities. The repressor activity of CtBP2 is reciprocally regulated by NADH and acyl-CoAs. CtBP2 represses Forkhead box O1 (FoxO1)-mediated hepatic gluconeogenesis directly as well as Sterol Regulatory Element-Binding Protein 1 (SREBP1)-mediated lipogenesis indirectly. The activity of CtBP2 is markedly defective in obese liver reflecting the metabolic perturbations. Thus, liver-specific CtBP2 deletion promotes hepatic gluconeogenesis and accelerates the progression of steatohepatitis. Conversely, activation of CtBP2 ameliorates diabetes and hepatic steatosis in obesity. The structure-function relationships revealed in this study identify a critical structural domain called Rossmann fold, a metabolite-sensing pocket, that is susceptible to metabolic liabilities and potentially targetable for developing therapeutic approaches

27. Unfolding is the driving force for mitochondrial import and degradation of the Parkinson's disease-related protein DJ-1

Queliconi BB, Kojima W, Kimura M, Imai K, Udagawa C, Motono C, Hirokawa T, Tashiro S, Caaveiro JMM, Tsumoto K, Yamano K, Tanaka K and Matsuda N.

Diverse genes associated with familial Parkinson's disease (familial Parkinsonism) have been implicated in mitochondrial quality control. One such gene, PARK7 encodes the protein DJ-1, pathogenic mutations of which trigger its translocation from the cytosol to the mitochondrial matrix. The translocation of steady-state cytosolic proteins like DJ-1 to the mitochondrial matrix upon missense mutations is rare, and the underlying mechanism remains to be elucidated. Here, we show that the protein unfolding associated with various DJ-1 mutations drives its import into the mitochondrial matrix. Increasing the structural stability of these DJ-1 mutants restores cytosolic localization. Mechanistically, we show that a reduction in the structural stability of DJ-1 exposes a cryptic N-terminal mitochondrial-targeting signal (MTS), including Leu10, which promotes DJ-1 import into the mitochondrial matrix for subsequent degradation. Our work describes a novel cellular mechanism for

targeting a destabilized cytosolic protein to the mitochondria for degradation.

28. Structural and thermodynamical insights into the binding and inhibition of FIH-1 by the N-terminal disordered region of Mint3

Ten T, Nagatoishi S, Maeda R, Hoshino M, Nakayama Y, Seiki M, Sakamoto T and Tsumoto K.

Mint3 is known to enhance aerobic ATP production, known as the Warburg effect, by binding to FIH-1. Since this effect is considered to be beneficial for cancer cells, the interaction is a promising target for cancer therapy. However, previous research has suggested that the interacting region of Mint3 with FIH-1 is intrinsically disordered, which makes investigation of this interaction challenging. Therefore, we adopted thermodynamic and structural studies in solution to clarify the structural and thermodynamical changes of Mint3 binding to FIH-1. First, using a combination of circular dichroism, nuclear magnetic resonance, and hydrogen/deuterium exchange-mass spectrometry (HDX-MS), we confirmed that the N-terminal half, which is the interacting part of Mint3, is mostly disordered. Next, we revealed a large enthalpy and entropy change in the interaction of Mint3 using isothermal titration calorimetry (ITC). The profile is consistent with the model that the flexibility of disordered Mint3 is drastically reduced upon binding to FIH-1. Moreover, we performed a series of ITC experiments with several types of truncated Mint3s, an effective approach since the interacting part of Mint3 is disordered, and identified amino acids 78 to 88 as a novel core site for binding to FIH-1. The truncation study of Mint3 also revealed the thermodynamic contribution of each part of Mint3 to the interaction with FIH-1, where the core sites contribute to the affinity (ΔG), while other sites only affect enthalpy (ΔH), by forming noncovalent bonds. This insight can serve as a foothold for further investigation of intrinsically disordered regions (IDRs) and drug development for cancer therapy.

29. Optimization of anti-ADAMTS13 antibodies for the treatment of ADAMTS13-related bleeding disorder in patients receiving circulatory assist device support

Ito T, Minamitani T, Hayakawa M, Otsubo R, Akiba H, Tsumoto K, Matsumoto M and Yasui T.

ADAMTS13 (a disintegrin-like and metalloproteinase with thrombospondin type-1 motif 13)-related bleeding disorder has been frequently observed as a life-threatening clinical complication in patients carrying a circulatory assist device. Currently, treatment modalities for the bleeding disorder are very limited and not always successful. To address the unmet medical need, we constructed humanized antibodies of mouse anti-ADAMTS13 antibody A10 (mA10) by using complementarity-determining region (CDR) grafting techniques with human antibody frameworks, 8A7 and 16E8. The characteristics of the two humanized A10 antibodies, namely A10/8A7 and A10/16E8, were assessed in vitro and in silico. Among the two humanized A10 antibodies, the binding affinity of A10/16E8 to ADAMTS13 was comparable to that of mA10 and human-mouse chimeric A10. In addition, A10/16E8 largely inhibited the ADAMTS13 activity in vitro. The results indicated that A10/16E8 retained the binding affinity and inhibitory activity of mA10. To compare the antibody structures, we performed antibody structure modeling and structural similarity analysis in silico. As a result, A10/16E8 showed higher structural similarity to mA10, compared with A10/8A7, suggesting that A10/16E8 retains a native structure of mA10 as well as its antigen binding affinity and activity. A10/16E8 has great potential as a therapeutic agent for ADAMTS13-related bleeding disorder.

30. Epitope-dependent thermodynamic signature of single-domain antibodies against hen egg lysozyme

Akiba H, Tamura H, Caaveiro JMM and Tsumoto K.

A substantial body of work has been carried out describing the structural features of the complex between single-domain antibodies (VHHs) and antigens, and the preeminence for epitopes located at concave surfaces of the antigen. However, the thermodynamic basis of binding is far less clear. Here, we have analysed the energetic profiles of five VHHs binding to the catalytic cleft or to a noncleft epitope of hen egg lysozyme. Various binding energetic profiles with distinctive enthalpic/entropic contributions and structural distribution of critical residues were found in the five antibodies analysed. Collectively, we suggest that from an energetic point of view the binding mechanism is influenced by the shape of the epitope. This information may be beneficial for the design of tailored epitopes for VHHs and their practical use.

31. B cell-intrinsic TBK1 is essential for germinal center formation during infection and vaccination in mice

Lee MSJ, Inoue T, Ise W, Matsuo-Dapaah J, Wing JB, Temizoz B, Kobiyama K, Hayashi T, Patil A, Sakaguchi S, Simon AK, Bezbradica JS, Nagatoishi S, Tsumoto K, Inoue JI, Akira S, Kurosaki T, Ishii KJ and Coban C.

The germinal center (GC) is a site where somatic hypermutation and clonal selection are coupled for

antibody affinity maturation against infections. However, how GCs are formed and regulated is incompletely understood. Here, we identified an unexpected role of Tank-binding kinase-1 (TBK1) as a crucial B cell-intrinsic factor for GC formation. Using immunization and malaria infection models, we show that TBK1-deficient B cells failed to form GC despite normal Tfh cell differentiation, although some malaria-infected B cell-specific TBK1-deficient mice could survive by GC-independent mechanisms. Mechanistically, TBK1 phosphorylation elevates in B cells during GC differentiation and regulates the balance of IRF4/BCL6 expression by limiting CD40 and BCR activation through noncanonical NF-κB and AKTT308 signaling. In the absence of TBK1, CD40 and BCR signaling synergistically enhanced IRF4 expression in Pre-GC, leading to BCL6 suppression, and therefore failed to form GCs. As a result, memory B cells generated from TBK1-deficient B cells fail to confer sterile immunity upon reinfection, suggesting that TBK1 determines B cell fate to promote long-lasting humoral immunity.

32. Structure-based screening combined with computational and biochemical analyses identified the inhibitor targeting the binding of DNA Ligase 1 to UHRF1

Kori S, Shibahashi Y, Ekimoto T, Nishiyama A, Yoshimi S, Yamaguchi K, Nagatoishi S, Ohta M, Tsumoto K, Nakanishi M, Defossez PA, Ikeguchi M and Arita K.

The accumulation of epigenetic alterations is one of the major causes of tumorigenesis. Aberrant DNA methylation patterns cause genome instability and silencing of tumor suppressor genes in various types of tumors. Therefore, drugs that target DNA methylation-regulating factors have great potential for cancer therapy. Ubiquitin-like containing PHD and RING finger domain 1 (UHRF1) is an essential factor for DNA methylation maintenance. UHRF1 is overexpressed in various cancer cells and down-regulation of UHRF1 in these cells reactivates the expression of tumor suppressor genes, thus UHRF1 is a promising target for cancer therapy. We have previously shown that interaction between the tandem Tudor domain (TTD) of UHRF1 and DNA ligase 1 (LIG1) di/trimethylated on Lys126 plays a key role in the recruitment of UHRF1 to replication sites and replication-coupled DNA methylation maintenance. An arginine binding cavity (Arg-binding cavity) of the TTD is essential for LIG1 interaction, thus the development of inhibitors that target the Arg-binding cavity could potentially repress UHRF1 function in cancer cells. To develop such an inhibitor, we performed in silico screening using not only static but also dynamic metrics based on all-atom molecular dynamics simulations, resulting in efficient identification of 5-amino-2,4-dimethylpyridine (5A-DMP) as a novel TTD-binding compound. Crystal structure of the TTD in complex with 5A-DMP revealed that the compound stably bound to the Arg-binding cavity of the TTD. Furthermore, 5A-DMP inhibits the full-length UHRF1:LIG1 interaction in Xenopus egg extracts. Our study uncovers a UHRF1 inhibitor which can be the basis of future experiments for cancer therapy.

<Group III>

1. Development of new methods for analyzing neural circuits in the retina

Neural circuits in the central nervous system are the basis of various higher-order brain functions. It is also true in case of retina. In the retina, six main classes of neural cells connect systematically to make up complex neural circuits. Characteristics of the retinal neural cell functions have been examined mainly by the electrophysiological methods and models of cell connectivity have been proposed. Morphological studies of the actual neural connection, which constitute the physiological properties of retinal neurons, have been desired. Until recently the only method to reveal the three-dimensional (3D) connectivity of actual neural cells morphologically was to collect ultrathin serial sections and observe them in transmission electron microscope (TEM). But the technical difficulties discouraged us from such a troublesome procedure. Recent progress in scanning electron microscope (SEM) equipment allowed us to develop a new method to observe ultrathin TEM sections in SEM (thin section scanning electron microscopy: TSSEM). To observe thin TEM sections, we have developed new sample staining methods to enhance electron contrast. To collect huge number of serial sections stably and efficiently, we have been developing new equipment and techniques. By using this equipment, it became possible to cut more than 1000 serial sections of less than 30 nm thickness much easier. We have analyzed about 500 serial thin sections of zebrafish retinal outer plexiform layer by this method and succeeded in tracing thin processes of bipolar cells into the photoreceptor terminals.

Aside from getting 3D information, TSSEM method provide us precise information of much wider areas of thin sections more effectively and more easily. Such studies are currently in progress.

2. Collaborative and supportive works as electron microscope core-laboratory

This group is also engaged in collaborative researches using electron microscope. We offer supports for the research projects those need electron microscopic analysis. The services available in this group are the conventional thin section transmission electron microscopy, immuno-electron microscopy, negative staining techniques and scanning electron mi-

croscopy. By using these individual technique or combination of some of these we can offer direct visual evidence that cannot be acquired by other methods. This year, 19 projects in 13 laboratories were performed as core-laboratory works.

a. Thin section transmission electron microscopy

Thin section transmission electron microscopy is the most widely used technique to observe the inner structure of cells and tissues. In this method, samples are fixed and embedded in epoxy resin, thin sections with about 70 nm thickness are cut and observed in the electron microscope. In case of immuno-electron microscopy, thin sections are obtained by similar procedure and the antigen epitopes exposed on the surface of the sections are marked by sequential reaction with appropriate primary antibodies and colloidal gold labeled secondary antibodies. This year, thin section electron microscopy and those combined with immuno-electron microscopy were used in many collaborative works.

a-1. Ultrastructural analysis of entry and assembly of Herpes Simplex Virus

We have been performing several studies with research groups in Dr. Kawaguchi[1]'s laboratory: [1]Division of Molecular Virology, Department of Microbiology and Immunology, regarding the infection/replication processes of herpes simplex virus (HSV). Thin section electron microscopy has been used to analyze the function of viral proteins in trans-nuclear membrane processes of the newly formed viruses. By analyzing the virus forming processes in some mutant host cells, we could analyze viral proteins as well as candidate host molecules those may be involved in the trans-nuclear process of the HSV.

a-2. Roles of membrane lipids in development and maintenance of photoreceptor outer-segment

We have been performing several studies also with research groups in Dr. Watanabe[2]'s laboratory: [2]Project Division of Molecular and Developmental Biology. This year, we analyzed the composition of phospholipids in individual cell types in developing mouse retina under physiological and pathological conditions and checked with electron microscopic data. With the combination of cell sorting and mass spectrometry analysis, most of phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) in retina are included in photoreceptor cells. When compared with the electron microscopic data in pathological conditions, PC and PE composition are dramatically changed before photoreceptor cell degeneration are apparent, suggesting that changes in PC and PE composition in photoreceptor cells may lead to the photoreceptor degeneration. (ref. Hamano *et al*) Another project about the structure of retina and retinal capillaly is also running with Dr. Watanabe's laboratory.

Some other collaborative research works using thin section electron microscopy and/or immuno-electron microscopy were performed with Dr. Sasou[3], [3]Division of Mucosal Immunology, Dr. Eguchi[4], in [4]Division of Genetics, Dr. Nakahara[5] in [5]Department of Life Science Dentistry, The Nippon Dental University and so on.

b. Negative staining techniques

Negative staining techniques are simple and quick method to observe the morphology of the macro-molecules. This year, negative staining techniques were used to analyze exosomes in collaboration with Dr. Hayashi[6] in [6]Division of Vaccine Science, Laboratory of Adjuvant Innovation. The same techniques are also used in the research with Dr. Shibata[7], and Dr. Samuel[7], in [7]Research Organization for Nano and Life Innovation, Waseda University. Negative staining techniques are also used to visualize virus like particles in the research with Dr. Kurokawa[3] and Dr. Yoshida[8] in [8]Project Division of Advanced Biopharmaceutical Science.

c. Conventional scanning electron microscopy

Conventional scanning electron microscopy is a technique used to examine the surface structure of the cells, tissues or other non-biological materials. Scanning electron microscopy combined with thin section transmission microscopy were used in collaborative work with Dr. Ishii[9], [9] Laboratory of Reproductive Systems Biology, about the structure of young and aged mouse oocyte zona pellucida.

Publications

<Group I>

Watanabe M, Arii J, Takeshima K, Fukui A, Shimojima M, Kozuka-Hata H, Oyama M, Minamitani T, Yasui T, Kubota Y, Takekawa M, Kosugi I, Maruzuru Y, Koyanagi N, Kato A, Mori Y, and Kawaguchi Y. Prohibitin-1 Contributes to Cell-to-Cell Transmission of Herpes Simplex Virus 1 via the MAPK/ERK Signaling Pathway. J Virol, 95: e01413-20, 2021.

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Research Center for Asian Infectious Diseases
アジア感染症研究拠点

Director/Professor	Yasushi Kawaguchi, D.V.M., Ph.D.	拠点長／教授	博士(獣医学)	川口　寧
Project Professor	Mitsue Hayashi, Ph.D.	特任教授	法学博士	林　光江
Visiting Professor	Masaki Imai, D.V.M., Ph.D.	客員教授	博士(獣医学)	今井正樹
Visiting Professor	Seiya Yamayoshi, D.V.M., Ph.D.	客員教授	博士(医学)	山吉誠也
Associate Professor	Akihisa Kato, Ph.D.	准教授	博士(医学)	加藤哲久
Project Associate Professor	Jin Gohda, Ph.D.	特任准教授	博士(薬学)	合田　仁
Project Senior Assistant Professor	Mizuki Yamamoto, Ph.D.	特任講師	博士(医学)	山本瑞生
Assistant Professor	Naoto Koyanagi, Ph.D.	助　教	博士(生命科学)	小柳直人
Assistant Professor	Yuhei Maruzuru, Ph.D	助　教	博士(生命科学)	丸鶴雄平

Research Center for Asian Infectious Diseases operates two project laboratories (one in Tokyo; one joint lab in Beijing) and a collaborative program (Harbin), supported by AMED, CAS, and CAAS. The center is conducting research on emerging and reemerging infections, aiming to translate its basic studies into practical use. And the project intends to train and educate young Japanese and Chinese scientists for the future generation.

BACKGROUND

China is an important neighbor of Japan, with geopolitical and economic interdependence. And it contains hot spots for emerging and reemerging infections, as exemplified by the occurrence of SARS coronavirus that shocked the world in 2003 and endemic avian influenza virus occasionally jumping from bird to human. The carrier rate of hepatitis viruses is very high and HIV infection is rapidly increasing. In the early 2000's the Institute of Medical Science, the University of Tokyo, (IMSUT) was looking for appropriate counterparts in China to strengthen the studies of emerging and reemerging infections.

IMSUT initially established three collaboration sites in fiscal 2005 in China, two in Beijing and one in Harbin, and had been conducting China-Japan research collaboration, for two 5-year terms (fiscal 2005-2010; 2010-2015), supported by the Ministry of Education, Culture, Sports, Science and Technology under the directorship of Aikichi Iwamoto, former project director. IMSUT thus set up a new sustainable system that allowed IMSUT scientists to work in China, along with Chinese scientists, focusing on the studies of emerging and reemerging infections. In 2015 Yasushi Kawaguchi succeeded A. Iwamoto as project director and launched the project *China-Japan Research Collaboration on Defense against Emerging and Reemerging Infections*, a 5-year J-GRID program of Japan Agency for Medical Research and Development (AMED). In 2020 based on the results of the previous five years, he launched another project *Studies to Control Emerging, Re-emerging and Imported Infectious Diseases to Be Conducted in International Collaboration Sites in China* under a 5-year AMED program *Japan Program for Infectious Diseases Research and Infrastructure*.

In 2005 IMSUT had founded two joint laboratories in collaboration with the Institute of Biophysics (IBP) and Institute of Microbiology (IM), which belong to the Chinese Academy of Sciences (CAS), a large national institution consisting of more than 100 research institutes all over China. IMSUT has dispatched Jin Gohda to IM as a principal investigator (PI). Along with his Chinese staffs, PI is conducting basic and translational studies of HIV, MERS coronavirus, dengue virus and SARS-CoV-2. In 2015 IMSUT has set up another project laboratory in Tokyo, whose studies complement those in Beijing. IMSUT is also conduct-

ing a joint research program on avian influenza virus between Yoshihiro Kawaoka at IMSUT and Hualan Chen at the Harbin Veterinary Research Institute (HVRI) of Chinese Academy of Agricultural Sciences. The activities in Beijing and Harbin are supported by Mitsue Hayashi of the Beijing Project Office.

This project, making the most of the opportunity of collaboration with the highly advanced Chinese institution, aims to translate our basic studies into practical use in future. During the course of the collaboration the project intends to train and educate young Chinese and Japanese scientists for the future generation and hopes to contribute to the friendship between the two peoples.

PROJECT LABORATORIES AND PROGRAM

Y. Kawaguchi (Director of Research Center for Asian Infectious Diseases; Project Director) manages the Center and the AMED-supported Project, which includes the domestic and overseas laboratories and program. He coordinates their activities and decides the direction of research. He and his group conduct studies of molecular virology and immunology of herpes virus in the Research Center for Asian Infectious Diseases.

a. Project Laboratory at IMSUT and Joint Laboratory at IMCAS

Many enveloped viruses, such as HIV-1, flavivirus, herpes simplex virus, and coronavirus, are pathogenic and of clinical importance. J. Gohda's and Y. Kawaguchi's groups are conducting a basic research on the development of antiviral therapy for infectious diseases caused by enveloped viruses.

Severe acute respiratory syndrome coronavirus 2 (SRAS-CoV-2) is the causative virus of Coronavirus disease 2019 (COVID-19), which has spread worldwide since the first case was reported in China in December 2019. The rapid development of antiviral drugs and vaccine against SARS-CoV-2 infection is needed for bringing an ongoing pandemic of COVID-19 to an end. J. Gohda and his group established a dual split protein-based cell fusion assay for SARS-CoV-2 spike protein to evaluate the antiviral activities of compounds and antibodies against SARS-CoV-2. Last year, we found by using the fusion assay that an existing Japanese pancreatitis drug, nafamostat strongly prevents viral entry of SARS-CoV-2 by inhibiting a serine protease, TMPRSS2, which is crucial for membrane fusion between SARS-CoV-2 and its target cells. This year, we performed chemical library screening using the fusion assay to identify a new viral entry inhibitor of SARS-CoV-2 and found that several compounds inhibit virus-cell fusion in TMPRSS2-dependent or -independent manners. These compounds might lead to the development of an antiviral drug against SARS-CoV-2 infection. Furthermore, we found that metalloproteinases, including a disintegrin and metalloproteinase 10 (ADAM10), are specifically involved in SARS-CoV-2 viral entry in some cell-types. The metalloproteinase-dependent viral entry induced syncytia formation and a cytopathic effect of SARS-CoV-2-infected cells in a TMPRSS2-independent manner. In addition, marimastat and prinomastat, which are clinically tested metalloproteinase inhibitors, effectively blocked the metalloproteinase-mediated SARS-CoV-2 infection. These results suggest not only that the metalloproteinase-dependent viral entry of SARS-CoV-2 might contribute to the complex pathogenesis unique to COVID-19, but that metalloproteinase inhibitors might lead to the development of effective therapies.

The use of combination anti-retroviral therapy (cART) has considerably contributed to preventing the development of AIDS in patients infected with human immunodeficiency virus type 1 (HIV-1). However, HIV-1 latent reservoirs harboring silenced but replication-competent provirus are a major obstacle against viral eradication in the infected patients. The "shock and kill" strategy, which is one of promising approaches to a cure of HIV-1 infection, is aimed to reactivate the latent provirus by treatment with latency reversing agents (LRAs), which is called "shock", in the presence of antiretroviral drugs. Some drugs have been so far identified as an LRA. However, no drugs that cause cell death of HIV-1 latent reservoirs, which is called "kill", has not been identified yet. J. Gohda and his group are currently trying to identify compounds that block the release of HIV-1 viral particles from infected cells. These compounds are expected to "kill" the reactivated reservoirs by a cytopathic effect through accumulation of cytotoxic viral proteins in the cells without releasing infectious viral particles. Last year, we established the detection system for quantification of HIV-1 viral particles by using an HIV-1 vector with HiBiT-sequence inserted in a gag gene. In this system, viral particles in culture supernatant of infected cells with HIV-1 derived from the vector can be easily detected and quantified by a NanoLuc assay. We now identified several drugs that probably block the release of viral particles, through drug library screening using the system.

b. Joint Laboratory at IBPCAS

The Joint Laboratory at IBPCAS was closed in March 2020. However, the research collaboration and academic exchange between IMSUT and IBPCAS is still ongoing.

c. Collaborative research program with HVRI

At the end of 2019, a novel coronavirus (severe acute respiratory syndrome coronavirus 2; SARS-CoV-2) was detected in Wuhan, China, that spread rapidly around the world, with severe consequences for human health and the global economy. In China,

highly pathogenic avian influenza (HPAI) H5N1 virus transmitted to humans in 1997; since 2013, low pathogenic avian influenza A H7N9 viruses have caused sporadic infections in humans; and in 2016, HPAI H7N9 viruses emerged raising concerns of a pandemic. For these reasons, HVRI (Director, Zhigao Bu) has been conducting collaborative research on influenza virus, SARS-CoV-2, and other emerging viruses from all over Asia.

HVRI focuses on avian influenza viruses that are circulating in Chinese wild waterfowl, domestic poultry, and swine. Specifically, Y. Kawaoka and his group study type A influenza viruses and SARS-CoV-2 viruses, with an emphasis on viral pathogenicity in various hosts, viral evolution, and viral surveillance.

The two major findings this year are: (1) In early 2021, a new variant of SARS-CoV-2, designated P.1, emerged in Brazil. We characterized a P.1 variant isolated in Japan by using Syrian hamsters. In hamsters, the variant showed replicative abilities and pathogenicity similar to those of early and contemporary strains. Sera and/or plasma from convalescent patients and BNT162b2 messenger RNA vaccinees showed comparable neutralization titers across the P.1 variant and contemporary strains. In contrast, the contemporary strains were less well recognized than the P.1 variant by serum from a P.1-infected patient. Prior infection with contemporary strains efficiently prevented the replication of the P.1 variant in the lower respiratory tract of hamsters upon reinfection. These findings suggest that the P.1 variant may be somewhat antigenically different from the early and contemporary strains of SARS-CoV-2. (2) We performed a longitudinal study of antibody responses against SARS-CoV-2 in symptomatic patients. Sequential blood samples were collected from 39 individuals at various timepoints between 0 and 154 days after onset. The IgG titers to the receptor binding domain (RBD) of the S protein, the ectodomain of the S protein, and the N protein peaked at about 20 days after onset, gradually decreased thereafter, and were maintained for several months after onset. Extrapolation modeling analysis suggested that the IgG antibodies were maintained for this amount of time because the rate of reduction slowed after 30 days post-onset. IgM titers to the RBD decreased rapidly and disappeared in some individuals after 90 days post-onset. All patients, except one, possessed neutralizing antibodies against authentic SARS-CoV-2, which they retained at 90 days after onset. The highest antibody titers in patients with severe infections were higher than those in patients with mild or moderate infections, but the decrease in antibody titer in the severe infection cohort was more remarkable than that in the mild or moderate infection cohort. Our results show that the antibody response against the first SARS-CoV-2 infection in symptomatic patients is typical of that observed in an acute viral infection.

IMSUT PROJECT OFFICE

The office (M. Hayashi) supports the activities of the joint laboratory in Beijing and the joint research program in Harbin. It serves as Secretariat for Steering Committee Meeting and files MOU and Minutes. It helps scientists visiting the joint laboratory/program for collaborative research. It has been gathering the information about emerging infections in China from the Chinese mass media and official announcements, and the gathered information (in Japanese) has been presented and updated on the website of the Project (http://www.rcaid.jp/).

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Laboratory of Molecular Genetics (Frontier Research Unit)
遺伝子解析施設（フロンティア研究領域）

Professor	Yuji Yamanashi, Ph.D.	教　授　理学博士	山　梨　裕　司
Associate Professor	Kazuo Tatebayashi, Ph.D.	准教授　博士（薬学）	舘　林　和　夫

The Laboratory of Molecular Genetics was established for developing various molecular genetic techniques, spreading them to IMSUT investigators and supporting security management related to experiments carried out using recombinant DNA technologies. Since 2017, this laboratory has integrated the Frontier Research Unit for supporting selected investigators to challenge new fields of bio-medical sciences.

Frontier Research Unit

Protein phosphorylation and dephosphorylation are among the most important intracellular signaling mechanisms, and are mediated, respectively, by protein kinases and protein phosphatases. We study various aspects of cellular signal transduction with a particular emphasis on the role and regulation of protein phosphorylation and dephosphorylation in cellular stress responses, using yeast cells.

1. Osmostress enhances Pbs2 MAP2K phosphorylation by Ssk2/22 MAP3K in addition to acting on the membrane-associated osmosensors and Hog1 MAPK in the yeast osmoregulatory HOG pathway

Kazuo Tatebayashi

The family of mitogen-activated protein kinases (MAPKs) are major intracellular signal transducers in eukaryotic cells and are associated with many human diseases. Each MAPK is activated in a three-tiered kinase cascade composed of a MAPK kinase kinase (MAPKKK or MAP3K), a MAPK kinase (MAPKK or MAP2K), and a MAPK. Activated MAP3K activates a cognate MAP2K by phosphorylating two conserved residues in the flexible activation loop of the MAP2K. In turn, an activated MAP2K activates a cognate MAPK by phosphorylating the conserved threonine and tyrosine residues in the latter's activation loop. A MAPK signal transduction pathway commonly comprises, in addition to the kinase cascade, an upstream receptor or sensor, and downstream MAPK substrates.

The yeast MAPK Hog1 is activated by hyperosmotic stress through the High Osmolarity Glycerol (HOG) pathway, and orchestrates an array of osmo-adaptive changes in transcription, translation, cell cycle, and metabolism. The current widely held model of the HOG pathway is as follows. The upstream portion of the HOG pathway is comprised of the functionally redundant SHO1 and SLN1 branches. In the SHO1 branch, osmosensing complexes composed of Sho1, Opy2, Hkr1, and Msb2 activate the MAP3K Ste11. In the SLN1 branch, the Sln1-Ypd1-Ssk1 phospho-relay mechanism is involved in activation of the functionally redundant MAP3Ks Ssk2 and Ssk22 (Ssk2/22). Ste11 and Ssk2/22 phosphorylate the MAP2K Pbs2 at Ser-514 and/or Thr-518. Phosphorylated Pbs2 then activates Hog1. Recently, we found that osmostress not only activates membrane-associated osmosensors, but also enhances Hog1 phosphorylation by mono-phosphorylated Pbs2. The lack of the osmotic enhancement of the Pbs2-Hog1 reaction suppresses Hog1 activation by basal MAP3K activities and prevents pheromone-to-Hog1 crosstalk in the absence of osmostress, which ensures the appropriate Hog1 activation only under high osmolarity.

This year, we demonstrated that osmostress acts on the process of Pbs2 phosphorylation by Ssk2/22 in the SLN1 branch, as well as the membrane-associated

osmosensors and Hog1. It was believed that the inhibition of the Sln1-Ypd1-Ssk1 phospho-relay by osmostress leads to activation of the downstream Ssk2/22 MAP3K and Pbs2 MAP2K. However, the deletion of the *SLN1* gene, thus inhibiting the phospho-relay, resulted in only weak phosphorylation of Pbs2, which was further elevated to a full level upon osmotic stress. These results indicate that the Ssk2/22-Pbs2 reaction is osmotically enhanced as Hog1 is. The underlying mechanism is under study.

2. The type IIC protein phosphatase Ptc1 negatively regulates the yeast osmo-regulatory HOG pathway by dephosphorylating Pbs2 MAP2K as well as Hog1 MAPK.

Kazuo Tatebayashi

The yeast osmo-regulatory HOG pathway is activated and inactivated through phosphorylation and dephosphorylation of the signaling molecules. Hog1 MAPK and Pbs2 MAP2K require phosphorylation in the activation loop for their activation. The tyrosine protein phosphatases Ptp2 and Ptp3 and the type IIC protein phosphatase Ptc1 were reported to negatively regulate the HOG pathway by dephosphorylating phospho-Tyr, and phospho-Thr in the Hog1 protein, respectively. Ptc1 is recruited to Pbs2-Hog1 complex via adaptor protein Nbp2 for dephosphorylation of Hog1. In spite of the indirect interaction of Pbs2 and Ptc1, it has remained unclear whether Pbs2 is dephosphorylated by Ptc1 for its negative regulation.

This year, we examined a possible involvement of Ptc1 in the dephosphorylation of Pbs2. Disruption of the *PTC1* gene led to constitutive phosphorylation in the activation loop in Pbs2 even in the absence of osmotic stress. Upon high osmolarity, the extent of Pbs2 phosphorylation was further elevated and its duration was prolonged in the *ptc1Δ* strain compared with those in the *PTC1*$^{+}$ strain. Furthermore, overexpression of Ptc1 significantly reduced the phosphorylation level of Pbs2 under high osmolarity. These results demonstrated that Ptc1 is the major phosphatase responsible for the dephosphorylation of Pbs2.

3. A novel multifunctional role for Hsp70 in binding post-translational modifications on client proteins.

Nitika[1], Bo Zheng[1], Linhao Ruan[2,3], Jake T. Kline[6], Jacek Sikora[7], Mara Texeira Torres[8], Yuhao Wang[2,3], Jade E. Takakuwa[1] Romain Huguet[9], Cinzia Klemm[8], Verónica A. Segarra[10], Matthew J. Winters[11], Peter M. Pryciak[11], Peter H. Thorpe[9], Kazuo Tatebayashi, Rong Li[2,3,4,5], Luca Fornelli[6] and Andrew W. Truman[1], [1]Department of Biological Sciences, The University of North Carolina at Charlotte, Charlotte, [2]Center for Cell Dynamics and Department of Cell Biology, Johns Hopkins University School of Medicine, [3]Biochemistry, Cellular and Molecular Biology (BCMB) Graduate Program, Johns Hopkins University School of Medicine, [4]Mechanobiology Institute and Department of Biological Sciences, National University of Singapore, Singapore, [5]Department of Chemical and Biomolecular Engineering, Whiting School of Engineering, Johns Hopkins University, [6]Department of Biology, University of Oklahoma, [7]AbbVie Inc., [8]School of Biological and Chemical Sciences, Queen Mary University, [9]Thermo Scientific, [10]Department of Biology, High Point University, [11]Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School

Hsp70 interactions are critical for cellular viability and the response to stress. Previous attempts to characterize Hsp70 interactions have been limited by their transient nature and inability of current technologies to distinguish direct vs bridged interactions. We report the novel use of cross-linking mass spectrometry (XL-MS) to comprehensively characterize the budding yeast Hsp70 protein interactome. Using this approach, we have gained fundamental new insights into Hsp70 function, including definitive evidence of Hsp70 self-association as well as multi-point interaction with its client proteins. In addition to identifying a novel set of direct Hsp70 interactors which can be used to probe chaperone function in cells, we have also identified a suite of post-translational modification (PTM)-associated Hsp70 interactions. The majority of these PTMs have not been previously reported and appear to be critical in the regulation of client protein function. These data indicate that one of the mechanisms by which PTMs contribute to protein function is by facilitating interaction with chaperones. Taken together, we propose that XL-MS analysis of chaperone complexes may be used as a unique way to identify biologically-important PTMs on client proteins.

Publications

Nitika, Bo Zheng, Linhao Ruan, Jake T. Kline, Jacek Sikora, Mara Texeira Torres, Yuhao Wang, Jade E. Takakuwa1 Romain Huguet, Cinzia Klemm, Verónica A. Segarra, Matthew J. Winters, Peter M. Pryciak, Peter H. Thorpe, Kazuo Tatebayashi, Rong Li, Luca Fornelli and Andrew W. Truman1 A novel multifunctional role for Hsp70 in binding post-translational modifications on client proteins. bioRxiv preprint doi: https://doi.org/10.1101/2021.08.25.457671

IMSUT Hospital

Department of Hematology/Oncology
血液腫瘍内科

Professor	Yasuhito Nannya, M.D., D.M.Sc.	教　授	博士(医学)	南谷泰仁
Project Professor	Satoshi Takahashi, M.D., D.M.Sc.	特任教授	博士(医学)	高橋　聡
Associate Professor	Yoichi Imai, M.D., Ph.D.	准教授	博士(医学)	今井陽一
Associate Professor	Tkiko Nagamura-Inue, M.D., Ph.D.	准教授	博士(医学)	長村登紀子
Project Associate Professor	Hiroshi Yasui, M.D., D.M.Sc.	特任准教授	博士(医学)	安井　寛
Assistant Professor	Takaaki Konuma, M.D., D.M.Sc.	助　教	博士(医学)	小沼貴晶
Assistant Professor	Tomohusa Fukuyama, M.D., D.M.Sc.	助　教	博士(医学)	福山朋房
Assistant Professor	Masamichi Isobe, M.D., D.M.Sc.	助　教	博士(医学)	磯部優理
Assistant Professor	Toyotaka Kawamata, M.D., D.M.Sc.	助　教	博士(医学)	川俣豊隆
Assistant Professor	Kazuaki Yokoyama, M.D., D.M.Sc.	助　教	博士(医学)	横山和明
Assistant Professor	Aki Sato, M.D., D.M.Sc.	助　教	博士(医学)	佐藤亜紀

We are conducting clinical, pathological, and therapeutic research on hematopoietic tumors and other hematological diseases. In the field of genomic medicine, which has been under development recently, research for clinical implementation is underway. In our laboratory, we have been conducting research on clinical sequencing in collaboration with HGC, as well as research on the automation and efficiency of curation and clinical implementation using artificial intelligence, and on the clinical significance of clinical sequencing. In addition, we are trying to understand the pathogenesis of genomic abnormalities revealed by clinical sequencing. In clinical transplantation, we have performed over 400 cord blood transplants and are promoting clinical research to optimize transplantation based on this data. Furthermore, we play a role as a clinical hub center for adult-onset histiocytosis, and we work on to introduce novel treatments into clinical practice for intractable adult-onset histiocytosis.

1. The impact of circulating tumor DNA status on acute myeloid leukemia and myelodysplastic syndromes with alloSCT: Interim results of a prospective study.

Ogawa M[1], Yokoyama K[1, 2], Yusa N[3], Kondo K[1], Takei T[1], Nakamura S[1], Ito M[1], Kasajima R[4], Yamamoto M[4], Shimizu E[5], Yamaguchi R[5], Imoto S[4], Takahashi S[1, 2], Miyano S[5], Tojo A[1, 2], Yasuhito N[1, 2] and Kanto Study Group for Cell Therapy.

[1] Division of Hematopoietic Disease Control

[2] Department of Hematology/Oncology, IMSUT Hospital

[3] Department of Applied Genomics, IMSUT Hospital

[4] Division of Health Medical Data Science

[5] Laboratory of Genome Database

[6] Division of Molecular Therapy

We previously reported the utility of residual circulating tumor DNA (ctDNA) status for identifying patients with acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) at high risk for relapse post myeloablative allogeneic hematopoietic

stem cell transplantation (alloSCT) in the retrospective setting. However, it remains to be elucidated whether this approach could be useful in the prospective setting as well. Here, we report the interim results from a Japanese multicenter prospective observational study, examining the clinical utility of this approach (KSGCT1702, conducted on behalf of Kanto Study Group for Cell Therapy). Since June 2018, we've enrolled patients with AML and MDS planning to undergo myeloablative alloSCT. We collected tumor and matched serum samples at diagnosis and serum samples every month until the fourth month post alloSCT. We subjected tumor DNA (bone marrow or peripheral blood), and buccal swab DNA, to next generation sequencing, identifying candidate driver mutations. We designed allele specific droplet digital PCR (ddPCR) assays for each to monitor ctDNA. The median detection limit of these assays was 0.04% (Nakamura et al, Blood 2019). The primary endpoint was to compare the cumulative incidence of relapse rate (CIR) within 1 year post alloSCT according to residual ctDNA status. The log-rank test was used for group comparison.

As of November 2019, a total of 38 patients were enrolled and 28 patients (26 AML and 2 MDS) of whom received alloSCT. The median follow up period post alloSCT was 119.5 (1-365) days. The median age was 54 years (25-66 years) including 15 males (53.6%). The conventional risk category was adverse or high risk in 42.9% of patients. To date, we've finished sequencing in 24 (85.7%) patients. A total of 33 somatic driver mutations/fusion genes were detected in all 24 patients, with one, two and three mutations found in 17, 5, and 2 individuals, respectively. These mutations included: single nucleotide variants, DNMT3A, CEBPA, NRAS, CEBPA, TET2, PTPN11, NPM1, IDH2, TP53, SMC3, RUNX1, JAK2, and FLT3; structural variants, CBFB/MYH11, KMT2A/MLLT3, and KMT2A/AFDN. We could construct ddPCR assays for 20 of 24 (83.3%) patients. Notably, there was a clear correlation of VAF between diagnostic ctDNA and matched tumor DNA from BM in available patients

($r2 = 0.86$; $p = 0.0009$). Of these 24 patients, one patient died without relapse, with six in clinical relapse with a median of 4 months (range, 1 to 10 months), and with the remaining 17 in remission. Of 20 patients available for personalized assays, fourteen patients were available for ctDNA status 3 months post allo-SCT, and 4 of whom were positive ctDNA status with the average allele frequency of 1.52 (0.12 to 5.0) %. Most importantly, when we compared CIR rate according to residual ctDNA status, positive ctDNA status at 3 months post alloSCT was associated with higher CIR at 10 months: 100% in positive patients vs. 0% in negative patients ($p = 0.0259$, Figure1). Our ctDNA monitoring could identify patients who were more likely to relapse. Additional enrollment of patients and further follow-up are needed to confirm this promising result.

2. Artificial intelligence (AI)-guided precision medicine approach to hematological malignancies.

Yokoyama K[1], Yokoyama N[2, 6], Nakamura S[3], Ogawa M[3], Takei T[3], Kobayashi M[3], Ando S[1], Kondo K[1], Mizusawa M[1], Isobe M[1], Tanoue S[1], Kawamata T[1], Makiyama J[1], Konuma T[1], Kato S[1], Imai Y[1], Takahashi S[1, 3], Shimizu E[4], Yamaguchi R[4], Imoto S[5], Furukawa Y[2, 6], Miyano S[4], Tojo A[1,7,] Yasuhito N [1,3]

[1] Department of Hematology/Oncology, IMSUT Hospital

[2] Department of Applied Genomics, IMSUT Hospital

[3] Division of Hematopoietic Disease Control

[4] Laboratory of Genome Database

[5] Division of Health Medical Data Science

[6] Division of Clinical Genome Research

[7] Division of Melecular Therapy

Next generation sequencing (NGS) of cancer genome is now becoming prerequisite for accurate diagnosis and proper treatment in clinical oncology (Precision oncology). While the genomic regions for NGS expand from a certain set of genes to whole exome or whole genome, the resulting sequence data becomes incredibly enormous, and then makes it quite laborious to translate the genomic data into medicine, so-called annotation and curation. We organized a clinical sequencing team and established a bidirectional (bed to bench and bench to bed) system to integrate clinical and genomic data in blood cancers. We also started a collaborative research with IBM Japan to adopt artificial or augmented intelligence (AI), Watson for Genomics (WG), to the pipeline of medical informatics. Genomic DNA was prepared from cancer cells as well as normal tissues (buccal swab) in each patient and subjected to NGS. Sequence data was analyzed using an in-house semi-automated pipeline in combination with WG, which was used to identify candidate driver mutations and relevant pathways, from which applicable drug information was deduced.

Until now, we have analyzed more than 400 patients in total with hematological malignancies including AML, MDS, MPN, et al., and could obtain many informative findings. Although actionable mutations are quite insufficient for clinical practice mainly due to the lack of available molecular-targeted agents, our preliminary results indicate that AI can be a promising support tool for precision medicine.

3. Prognostic value of measurable residual disease at allogeneic transplantation for adults with core binding factor acute myeloid leukemia in complete remission

Konuma, Takaaki[1], Kondo, Tadakazu[2], Atsuta, Yoshiko[3], Yanada, Masamitsu[4] the Adult Acute Myeloid Leukemia Working Group of the Japan Society for Hematopoietic Cell Transplantation

[1] Department of Hematology/Oncology, IMSUT Hospital

[2] Department of Hematology and Oncology, Graduate School of Medicine, Kyoto University

[3] Department of Healthcare Administration, Nagoya University Graduate School of Medicine

[4] Department of Hematology and Cell Therapy, Aichi Cancer Center

Pretransplant measurable residual disease (MRD) has been shown to be associated with relapse incidence following allogeneic hematopoietic cell transplantation (HCT) for acute myeloid leukemia (AML). However, it remains less clear whether pretransplant MRD status affects transplant outcomes in core binding factor AML (CBF-AML). We retrospectively evaluated the effect of pretransplant MRD, which was measured by a polymerase chain reaction of RUNX1-RUNX1T1 or CBFB-MYH11 fusion transcripts, on transplant outcomes for a cohort of 959 adult patients with t(8;21) or inv(16) AML treated by allogeneic HCT during complete remission (CR), between 2000 and 2018. Multivariate analysis showed the absence of pretransplant MRD was significantly associated with lower relapse (hazard ratio [HR], 0.46; P < 0.001), treatment failure (HR, 0.66; P = 0.004), and overall mortality (HR, 0.72; P = 0.037) among patients with t(8;21). However, pretransplant MRD negativity was not associated with relapse (HR, 0.73; P = 0.420), treatment failure (HR, 0.64; P = 0.063), or overall mortality (HR, 0.69; P = 0.149) among patients with inv(16). In subgroup analysis, pretransplant MRD status significantly affected relapse and LFS only in patients with t(8;21) undergoing allogeneic HCT during CR2. In conclusion, our data demonstrate the different prognostic values of pretransplant MRD for CBF-AML, highlighting the need to develop effective therapeutic strategies for such MRD-positive patients.

4. Single cord blood transplantation for acute myeloid leukemia patients aged 60 years or older: a retrospective study in Japan

Isobe, M.[1], Konuma, T.[1], Masuko, M.[2], Atsuta, Y.[3], Yanada, M.[4] Adult Acute Myeloid Leukemia Working Group of the Japan Society for Hematopoietic Cell, Transplantatio

[1] Department of Hematology/Oncology, IMSUT Hospital

[2] Department ofHematopoietic Cell Transplantation, Niigata University Medical and Dental Hospital,

[3] Department of Healthcare Administration, Nagoya University Graduate School of Medicine

[4] Department of Hematology and Cell Therapy, Aichi Cancer Center

The availability of alternative donor sources could allow elderly patients to receive allogeneic hematopoietic cell transplantation (HCT). We retrospectively evaluated the outcomes of single-unit cord blood transplantation (CBT) in 1577 patients aged >/=60 years with acute myeloid leukemia (AML) in Japan between 2002 and 2017. In total, 990 (63%) patients were not in complete remission (CR) at the time of CBT. A myeloablative conditioning regimen (52%) and calcineurin inhibitor (CI) + mycophenolate mofetil (MMF)-based graft-versus-host disease (GVHD) prophylaxis (45%) were more commonly used. With a median follow-up for survivors of 31 months, the probability of overall survival and the cumulative incidence of leukemia-related mortality at 3 years was 31% and 29%, respectively. The cumulative incidence of non-relapse mortality (NRM) at 100 days and 3 years were 24% and 41%, respectively. The cumulative incidences of grade II-IV and grade III-IV acute GVHD at 100 days and extensive chronic GVHD at 2 years were 44%, 16%, and 14%, respectively. The cumulative incidence of neutrophil engraftment was 80% at 42 days. Results of multivariate analysis indicated that the following factors were significantly associated with higher overall mortality: performance status >/=1, hematopoietic cell transplantation-specific comorbidity index >/=3, adverse cytogenetics, extramedullary disease at diagnosis, and non-CR status at CBT. By contrast, female sex, HLA disparities >/=2, mycophenolate mofetil-based GVHD prophylaxis, and recent CBT were significantly associated with lower overall mortality. In conclusion, single CBT offers a curative option for AML patients aged >/=60 years with careful patient selection.

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IMSUT Hospital

Department of Infectious Diseases and Applied Immunology
感染免疫内科

Associate Professor	Takeya Tsutsumi, M.D., D.M.Sc.	准教授	博士(医学)	堤　武也
Professor	Hiroshi Yotsuyanagi, M.D., D.M.Sc.	教　授	博士(医学)	四柳　宏
Project Assistant Professor	Michiko Koga, M.D., D.M.Sc	特任助教	博士(医学)	古賀道子
Assistant Professor	Eisuke Adachi, M.D., D.M.Sc.	助　教	博士(医学)	安達英輔
Assistant Professor	Makoto Saito, M.D., D.Phil.	助　教	博士(医学)	齋藤　真

Founded in 1981, IMSUT hospital started HIV clinic in 1986, and 1286 HIV-infected patients visited us by 2021. Currently, 556 patients in total are under our clinical management. Besides HIV infection, we have been treating patients with other infection such as hepatitis and malaria. Since the emergence of COVID-19, we started to treat COVID-19 patients, and about 570 patients admitted to our hospital by the end of 2021. (66/70 words)

1. Treatment of COVID-19 in IMSUT hospital

Eisuke Adachi, Makoto Saito[1], Hiroyuki Nagai, Michiko Koga[1], Lay Ahyoung Lim, Kazuhiko Ikeuchi, Takeya Tsutsumi[1], Hiroshi Yotsuyanagi[1]
[1] Division of Infectious Diseases, The Advanced Clinical Research Center, IMSUT

Since the beginning of 2020, there have been the outbreak of coronavirus disease 2019 (COVID-19) in Japan, and we started to treat COVID-19 patients at the IMSUT Hospital in February. There were about 570 patients admitted to the hospital by the end of 2021. In 2020, most (about 85%) of the patients had mild severity of COVID-19, but in 2021, we treated many (about 50%) patients with moderate or severe severity who needed oxygen administration. (Several patients became exacerbated during the hospitalization and were transferred to other hospitals.)

2. Treatment of HIV infection in IMSUT hospital: Statistical characteristics of HIV infected patients in IMSUT hospital this year

Michiko Koga[1], Eisuke Adachi, Lay Ahyoung Lim, Kazuhiko Ikeuchi, Makoto Saito[1], Tadashi Kikuchi, Takashi Odawara, Takeya Tsutsumi[1],Hiroshi Yotsuyanagi[1]

Sixteeen new patients with HIV-1 infection visited to our hospital this year (from January 1 to December 31, 2021), and 556 patients in total are under medical management in our outpatient clinic. The total number of HIV-infected in-patients during 2021 was 22. From the beginning of 2020, the burden of COVID-19 medical care was added, but the number of HIV-infected patients treated did not change (Fig. 1). Anti-retroviral therapy (ART) has been introduced to 553 HIV-infected patients in our hospital, and most of their HIV viral loads have been well controlled. After

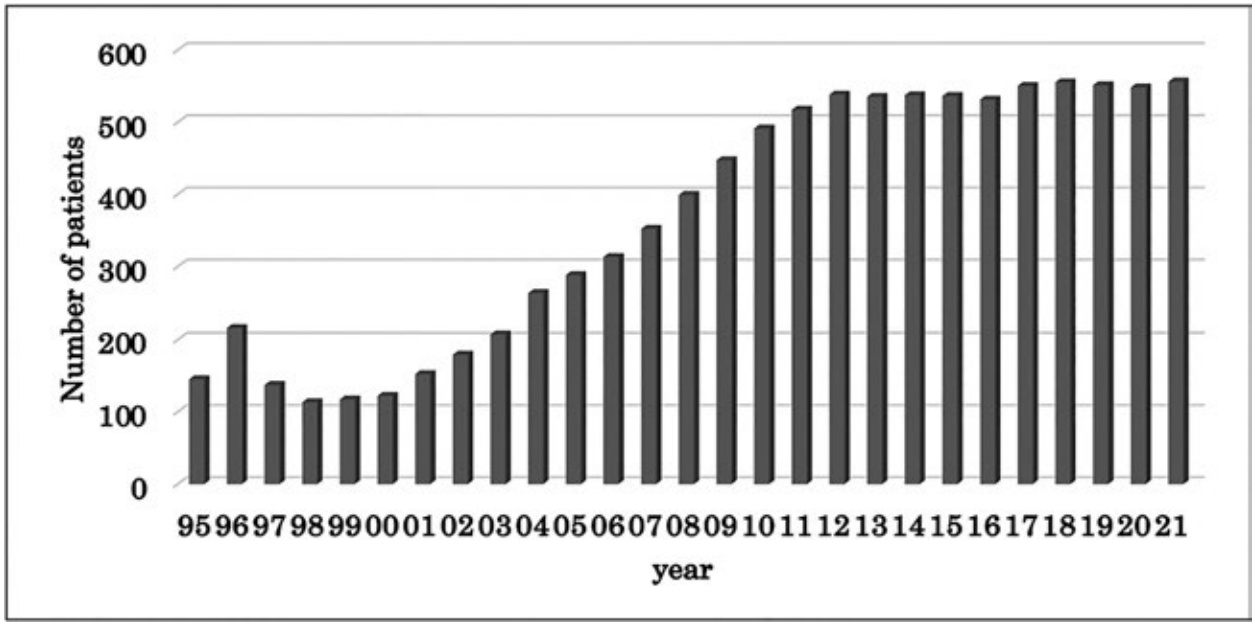

Figure 1. Number of HIV-infected outpatients in IMSUT Hospital

one year of ART, the viral loads become less than 100 copies/ml in 98.5% of HIV-infected patients in our outpatient clinic, presumably underscored by the change in the method of quantitative HIV-RNA assay. Consequently, the patients are able to maintain good condition as long as they keep excellent drug adherence rates. The clinical management of HIV-infected patients has been changing from how to treat opportunistic infections into how to control patients with ART.

3. Pre and post-travel treatment and clinical research of tropical diseases in IMSUT hospital

Makoto Saito[1], Eisuke Adachi, Michiko Koga[1], Kazuhiko Ikekuchi, Tadashi Kikuchi, Takashi Odawara, Takeya Tsutsumi[1], Hiroshi Yotsuyanagi[1]:

The pandemic of COVID-19 has had unprecedented impact of our life: global transport and travelling is one of the most affected areas. The decline of international travelling has changed our pre- and post-travel clinic. While pre-travel consultations and visits of returned travelers get fewer, there have been a huge demand of pre-travel PCR testing, which is required to enter majority of the countries. Our hospital started providing pre-travel PCR testing service in mid-May 2020, which was one of the earliest in Japan.

For the tropical and parasitic diseases, dozens of important medicines essential for treatment of them are not licensed in Japan. Research Group on Chemotherapy of Tropical Diseases, Research on Publicly Essential Drugs and Medical Devices, Grant from Japan Agency for Medical Research and Development had been established to cope with this situation. We are one of the designated medical institutions of the research group using these orphan drugs if needed, and colleting clinical data.

4. Treatment of hepatitis in IMSUT hospital:

Takeya Tsutsumi[1], Michiko Koga[1], Lay Ahyoung Lim, Kazuhiko Ikeuchi, Makoto Saito[1], Eisuke Adachi, Hiroshi Yotsuyanagi[1]

About 300 HIV-non-infected patients with liver diseases such as viral hepatitis and NAFLD are under medical management in our outpatient clinic. Several patients were introduced from outside for the treatment of chronic hepatitis C with direct acting anti-virals (DAA) and successfully achieved the sustained viral response (SVR). In addition, we treated HIV-infected patients who developed acute hepatitis C with DAAs, who finally achieved SVR.

5. Clinical trial of ebola virus disease vaccine, the booster SARS-CoV2 inactivated vaccine and research of influenza virus in IMSUT hospital

Michiko Koga[1], Lay Ahyoung Lim, Kazuhiko Ikeuchi, Makoto Saito[1], Eisuke Adachi, Takeya Tsutsumi[1], Hiroshi Yotsuyanagi[1]

The clinical study of the ebola virus disesase vaccine is progressive in cooperation with the Division of Virology and the Center for Translational Research in IMSUT hospital. And the influenza virus study has also proceeded in cooperation with the Division of Virology in IMSUT hospital. The clinical study of the booster SARS-CoV2 inactivated vaccine has started with the Division of Virology and the Division of Vaccine Science, the Center for Translational Research in IMSUT hospital.

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IMSUT Hospital

Department of Rheumatology and Allergy
アレルギー免疫科

Associate Professor Motohisa Yamamoto, M.D., D.M.Sc. 准教授 博士(医学) 山 本 元 久

Our department is founded in 2001 to tackle systemic autoimmune inflammatory diseases including rheumatoid arthritis, systemic lupus erythematosus, vasculitic syndromes, and IgG4-related disease. We provide patients personalized and evidence-based medical service. Moreover, we challenge cutting edge science of autoimmune, rheumatic and allergic diseases and novel treatments for patients with these disorders. As part of an elite teaching hospital, we also contribute to preparing the next generation of leading academic physicians, scientists and clinician-educators.

1. Clinical activities in IMSUT Hospital

Motohisa Yamamoto, Masaaki Uehara

Rheumatologists at our division provide state-of-the-art diagnosis and treatment for systemic autoimmune diseases (total number of patients were approximately 3,000 per year). Our physicians have active basic and clinical research projects and also are involved in training of rheumatology specialists.

Rheumatologic services offered at IMSUT Hospital include:

- Outpatient consultations
- Outpatient specialty care for patients with rheumatic diseases
- Hospital consultations
- Education on rheumatologic diseases and treatments
- Clinical trials

2. Establish of new registry for the patients with IgG4-related disease and development of novel diagnostic and therapeutic approaches for IgG4-related disease

Motohisa Yamamoto, Masaaki Uehara

IgG4-related disease is a new disease concept, established this century. As a chronic fibro-inflammatory disorder, IgG4-related disease is characterized by elevated serum levels of IgG4 and abundant infiltration of IgG4-bearing plasma cells into and fibrosis of the involved organs. Whether the disorder is an autoimmune disease remains unclear; nevertheless, consultation with rheumatologists regarding patients with IgG4-related disease is increasing owing to the various organ dysfunction involved and the abnormal immune responses observed. We tackle elucidating the pathogenesis of IgG4-related disease and developing novel treatments. At first, we established a new registry system for the patients with IgG4-related disease (TOMMOROW registry), and started to enroll IgG4-related disease patients. We cooperate with national policies, and also provide the data to Rare Disease Data Registry of Japan (RADDAR-J), which was established by AMED. We will organize the clinical figures of IgG4-related disease and develop more accurate diagnostic and therapeutic approach by a TOMORROW registry. Furthermore, using the obtained blood and tissue samples, we will carry out multi-omics analysis. We will link the results to the individual clinical data, and promote personalized medicine that predicts therapeutic response and prognosis using artificial intelligence. In order to achieve this, we are currently conducting RNA-Seq of

both salivary gland specimens and peripheral blood mononuclear cells, microbiome analysis of saliva, and analysis of the relationship between therapeutic response and HLA.

3. Development of AI-based diagnostic, therapeutic methods and prognostic algorithms for rheumatic diseases

Motohisa Yamamoto, Masaaki Uehara

Rheumatic diseases are currently diagnosed using patterned diagnostic criteria based on a combination of physical, hematological, and imaging findings. In addition, the therapeutic strategy for rheumatic diseases is decided after carefully considering the distribution and degree of disability. We have developed a diagnostic algorithm for IgG4-related disease based on clinical data collected in a multicenter collaboration. The subjects were 602 patients with IgG4-RD who visited the Institute of Medical Science, The University of Tokyo (IMSUT) Hospital, The University of Tokyo Hospital, Kanazawa University Hospital, Shinshu University Hospital, Kyoto University Hospital, and Sapporo Medical University Hospital. The analysis was performed using a decision tree and a random forest model. A dataset including two basic patient characteristics and 29 laboratory findings was created for each case at the first visit. Both analysis showed good accuracy, sensitivity, and specificity of the algorithm. Algorithms for predicting response to therapy, complications, and prognosis are currently being developed for rheumatoid arthritis and other rheumatic disorders.

4. Development of preventive methods for glucocorticoid-induced myopathy and osteonecrosis

Motohisa Yamamoto, Masaaki Uehara

The administration of glucocorticoids to patients with rheumatic diseases often results in glucocorticoid-induced myopathy. We previously found that administration of branched-chain amino acids (BCAA) to such patients improves the loss of skeletal muscle, especially slow-twitch muscle. We also found that the serum concentration of the specific amino acids reflect to the slow-twitch muscle improvements. Based on this, we propose the need for separate muscle recovery methods for slow- and fast-twitch muscles, and investigate for the best method for each.

On the other hand, when a large amount dose of glucocorticoid is used for remission induction, the risk of osteonecrosis of the femoral head occurs. Currently, osteonecrosis of the femoral head is one of the complications that there is no way to prevent. In collaboration with the Department of Orthopaedic Surgery, Sapporo Medical University School of Medicine, we are working to develop a method to prevent osteonecrosis of the femoral head. Currently, several candidate drugs have been identified and clinical trials have been completed.

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IMSUT Hospital

Department of Oncology and General Medicine

腫瘍・総合内科

Head, Professor	Narikazu Boku, M.D., D.M.Sc.	教　授	博士(医学)	朴　　成和
Professor	Hiroshi Yotsuyanagi, M.D., D.M.Sc.	教　授	博士(医学)	四　柳　　宏
Associate Professor	Takeya Tsutsumi, M.D., D.M.Sc.	准教授	博士(医学)	堤　　武也
Associate Professor	Yoshihiro Hirata, M.D., D.M.Sc.	准教授	博士(医学)	平　田　喜　裕
Senior Assistant Professor	Yasuo Matsubara, M.D., D.M.Sc.	講　師	博士(医学)	松　原　康　朗
Project Senior Assistant Professor	Yasuki Hijikata, M.D., D.M.Sc	特任講師	博士(医学)	土　方　康　基
Project Senior Assistant Professor	Koichi Kimura, M.D., D.M.Sc.	特任講師	博士(医学)	木　村　公　一

The division of oncology and general medicine has been newly refounded in July 2021 taking over the department of general medicine. Our aim is to practice total human medical care including cancer patients in collaboration with other departments at IMSUT hospital and conduct exploratory clinical research. The members specialize in medical oncology, gastroenterology, hepatology, oncology, cardiology, endocrinology/metabolism. We have just started our new clinical trials.

1. Treatment of patients with advanced cancer.

Boku N.

Patients with various, mainly gastrointestinal, cancers were treated by standard therapy including chemotherapy, molecular target drugs, immune checkpoint inhibitors, in combination with surgery and radiation therapy. For some of them, optimal anti-cancer agents were recommended based on the next-generation sequencing. By the help of special patient support team undergirded by conference weekly, the quality and system of patient care has been improved. The number of chemotherapy cases at outpatient clinic has been doubled compared with that before July 2021. We join in the collaborative study group (West Japan Oncology Group), and we participate in several multi-center clinical trials in collaboration with other hospitals. A phase III trial for approval of ipilimumab plus nivolumab and cytotoxic agents will be started in January 2022. We contribute the publishing treatment guidelines such as gastric cancer, futility preservation and prevention of chemotherapy induced emesis. We are now planning a new project of "adaptive precision medicine" recommending an optimal molecular target drug according to the comprehensive phosphoproteomic information obtained from endoscopic biopsy before and after drug administration.

2. Treatment of drug-resistant *Helicobacter pylori* infection

Matsubara Y., Hirata Y.

Some patients fail to respond first- and second-line *Helicobacter pylori* (*H. pylori*) eradication therapy, but third-line eradication is not always done. Meanwhile, penicillin allergy patients do not take routine eradication medicines because insurance coverage regimens

in Japan include penicillin. In *H. pylori* out-patient clinic of IMSUT hospital, we make correct diagnosis of infection by multiple modalities, give eradication therapy for these refractory patients, and achieve high rates of successful eradication.

3. Endoscopic examination in IMSUT Hospital (Department of General Medicine)

Matsubara Y., Hirata Y.

About 700 cases of upper gastrointestinal endoscopy and about 500 cases of colonic endoscopy were performed from January 1 to December 31, 2021, while examinations were restricted due to covid-19. Furthermore, we participated in endoscopic health checkup in Minato Ward. We have diagnosed relatively rare disease (e.g. infectious disease, malignancy, other disease) in patients with immune dysfunction. We also performed prospective studies: 1) a study collaborated with Yamaguchi University, in which the fecal immunochemical test for hemoglobin, fecal DNA testing of TWIST1 methylation, and colonoscopy were performed on patients with or without colorectal neoplasia. 2) a study in which immune microenvironment was analyzed histochemically in colon adenomatous neoplasia.

4. Abdominal ultrasonography in IMSUT Hospital

Tsutsumi T

Four hundred forty-five cases of abdominal ultrasonography were performed from January to December in 2021. Pathologically abnormal lesions were detected such as liver tumors or pancreas tumors in some cases who were subsequently diagnosed as hepatocellular carcinoma or pancreas cancer. We also performed Fibroscan® for 271 cases with viral hepatitis or HIV infection.

5. Diagnosis and management of patients with genetic vascular diseases.

Takayuki Morisaki

Patients and family members with genetic vascular diseases including connective tissue disorders like Marfan syndrome, Loeys-Dietz syndrome and related diseases were diagnosed by taking their history, physical examination, imaging including echo-cardiography and genetic examination. These patients were followed-up and managed also by doctors in other medical institutions. Study to identify novel pathogenic genes for genetic vascular diseases was being performed.

6. Cardiac examinations in IMSUT Hospital

Kimura K., Morisaki T.

From January to December 2021, about 1500 cases of electrocardiogram, about 500 cases of echocardiography, and about 120 cases of Holter electrocardiogram were performed at IMSUT hospital, while examinations were restricted due to covid-19 pandemic.

7. Multicenter clinical studies and animal experiments regarding cardiomyopathy.

Kimura K.

We performed several clinical studies regarding echocardiography in collaboration with The University of Tokyo Hospital. Multicenter clinical studies were performed in collaboration with National Hospital Organization hospitals; Sendai-nishitaga National Hospital (Miyagi), Niigata National Hospital (Niigata), Shimoshizu National Hospital (Chiba), Hakone National Hospital (Kanagawa), Osaka-toneyama Medical Center (Osaka), and Hiroshima-nishi Medical Center (Hiroshima). We performed several animal experiments using CRISPR/Cas9 gene-edited rats in collaboration with Graduate School of Agricultural and Life Sciences, The University of Tokyo (Tokyo) and Cellular and Molecular Biotechnology Research Institute, National Institute of Advanced Industrial Science and Technology (Ibaraki). Other animal experiments using disease models of mice and dogs were performed in collaboration with National Center of Neurology and Psychiatry (Tokyo).

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IMSUT Hospital

Department of Applied Genomics
ゲノム診療科

Professor	Yoichi Furukawa, M.D., Ph.D.	教　授　博士(医学)	古　川　洋　一
Associate Professor	Tsuneo Ikenoue, M.D., Ph.D.	准教授　博士(医学)	池　上　恒　雄

Our department has been working on the application of human genome information in clinics. As clinical services in IMSUT Hospital, we provide genetic counseling, genetic tests for human malignancies such as leukemia and cancer, and a surveillance program for patients with hereditary colorectal cancer. In addition, we have been carrying out two research projects; 1) determination of genetic alterations in human tumors, and elucidation of the mechanisms underlying their development, and 2) clinical sequence for the implementation of genomic medicine

1. Genetic test of human neoplasms

Nozomi Yusa, Yoichi Furukawa

As a part of clinical service, we have performed genetic analysis of human neoplasms such as leukemia and colorectal cancer. In 2021, a total of 533 genetic analyses were performed in our department. The results were utilized for the precise classification of neoplasms, evaluation of disease status, selection of therapeutic drugs, and evaluation of the response to treatment.

2. Genetic counseling and related activities

Yoichi Furukawa, Yoshinori Murakami, Yataro Daigo, Tsuneo Ikenoue, Koichiro Yuji, Makoto Hirata, Reiko Sada, Mitsuko Nakazawa, Momoyo Ohki[1], Yoshinari Miyamoto[2], Masae Ono[3], Masahiko Suzuki[4], Mayumi Tamari[4], Toshihiro Tanaka[5], Shiro Ikegawa[6], Hidewaki Nakagawa[6], Natsuko Watanabe[7], Ai Yoshihara[7], Toru Akiyama[8]: [1]Bunkyo University, [2]National Center for Global Health and Medicine, [3]Tokyo Teishin Hospital, [4]Jikei Medical University, [5]Tokyo Medical and Dental University, [6]Center for Integrative Medical Sciences, RIKEN, [7]Ito Hospital, [8]Jichi Medical University.

We provided genetic counseling and genetic tests to clients who visited our counseling clinic. In 2021, we had a total of 14 counseling cases including hereditary breast and ovarian cancer, colonic polyposis, juvenile onset gastric cancer, tuberous sclerosis, facioscapulohumeral muscular dystrophy, and FG syndrome. In the counseling, we provided appropriate information about hereditary diseases to the clients and took their psychological care in collaboration with a clinical psychologist. Genetic testing was performed in cases with informed consent after thoughtful discussion about its merit and demerit.

Systematic surveillance programs are provided for the clients susceptible for hereditary tumors.

3. Application of liquid-based genetic diagnosis for the screening of endometrial cancer

Kiyoko Takane[1], Kiyoshi Yamaguchi[1], Tsuneo Ikenoue, Yoichi Furukawa
[1]Division of Clinical Genome Research, Advanced Clinical Research Center

We have conducted a study to elucidate the usefulness of liquid-based genetic diagnosis (LBGDx) for screening of endometrial cancer (EC) in collaboration with Department of Obstetrics and Gynecology, Sapporo Medical University. Although liquid-based cy-

tology (LBC) has increased the sensitivity of cytological diagnosis of EC compared with conventional smear cytology, the sensitivity of LBC for the detection of EC is between 70% and 96% and remains unsatisfactory. To investigate the efficacy of genetic testing in the screening of EC, we analyzed pathogenic mutations in a total of 195 LBC samples including 35 ECs by target sequencing using Cancer Hotspot Panel comprising of 50 cancer-related genes. As a result, we identified pathogenic mutations in 28 of the 35 ECs, showing the sensitivity of 75%. On the other hand, the sensitivity of endometrial cancer by LBC alone was 69% (24/35 ECs). Importantly, LBGDx detected seven out of ten EC cases that were negative for cytology. These results suggest that LBGDx should contribute to the increase of sensitivity in the screening of EC.

4. Clinical sequencing for the implementation of genomic medicine

Kiyoko Takane, Kiyoshi Yamaguchi, Tsuneo Ikenoue, Yoichi Furukawa, Eigo Shimizu[1], Rika Kasajima[1], Kotoe Katayama[1], Seiya Imoto[1], Tetsuo Shibuya[2], Kazuaki Yokoyama[3], Yasuhito Nanya[3], Koichiro Yuji[4], Rui Yamaguchi[5], Satoru Miyano[6]: [1]Division of Health Medical Intelligence, [2]Division of Medical Data Informatics, Human Genome Center, [3]Department of Hematology/Oncology, [4]Project Division of International Advanced Medical Research, Advanced Clinical Research Center, IMSUT, [5]Division of Cancer Systems Biology, Aichi Cancer Center Research Institute, [6]Systems Biology for Intractable Diseases, Medical Research Institute, Tokyo Medical and Dental University

The application of Next-Generation Sequencing (NGS) technology in clinical medicine has revolutionized molecular diagnostics by enabling multiple gene testing, or analysis of the entire exon or whole genome with a limited amount of DNA. In collaboration with Human Genome Center and Advanced Clinical Research Center, we have been working on the genetic diagnosis of patients with suspected hereditary cancer predisposition, and the implementation of precision medicine for patients with rare or intractable cancer.

We have applied NGS technology for molecular diagnostics of hereditary colon cancer syndromes such as familial adenomatous polyposis (FAP), polymerase proofreading-associated polyposis (PPAP), and Lynch syndrome (LS). LS, also known as hereditary nonpolyposis colorectal cancer syndrome (HNPCC), is the most common cause of hereditary colon cancer. Germline variants in the mismatch repair (MMR) genes are responsible for the disease. In the collaborative study with the Japanese Society for Colorectal Cancer (JSCCR), we previously identified substantial number of structural variations (SVs) in the MMR genes. Since detection of SVs using short-read NGS is a challenging work, we took advantage of long-read sequencing technology using Oxford Nanopore MinION device, and further tested the strategy of long-read sequencing coupled with hybridization-based enrichment system for the efficient and accurate detection of breakpoints. We selected four test cases – three with deletion ranging from 1.2 kb to 109.2 kb and one with 8.5 kb duplication in the MMR genes. This approach successfully detected all these SVs with accurate positions of the breakpoints. In addition, we newly identified a deletion across an 84 kb region of *MSH2* in a LS patient without pathogenic single nucleotide variants. These data suggest that long-read sequencing will help the identification of pathogenic SVs in patients with hereditary diseases.

We have been also working on the implementation of genomic data in clinics. An outpatient clinic service in IMSUT hospital offered the consultation of patients with rare or intractable cancer. Patients with colorectal, breast, uterine, pancreatic cancer, and angiosarcoma gave written informed consent for genetic analysis and prediction of treatment using artificial intelligence were enrolled in this study. Genetic alterations in their tumors were determined by NGS, and the data were subsequently analyzed by QIAGEN Clinical Insights (QCI). The results of QCI including predicted driver mutations and suggested actionable drugs were discussed in the Tumor Board which consists of physicians, medical oncologists, genetic counsellors, geneticists, bioinformaticians, and experts of ethics.

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IMSUT Hospital

Department of Radiology
放射線科

Associate Professor	Hiroyuki Akai, M.D., D.M.Sc.	准教授	博士(医学)	赤井宏行
Senior Assistant Professor	Toshihiro Furuta, M.D., D.M.Sc.	講師	博士(医学)	古田寿宏
Assistant Professor	Masaru Kamitani, M.D.	助教		神谷勝
Project Assistant Professor	Haruto Sugawara, M.D., D.M.Sc.	特任助教	博士(医学)	菅原暖斗

Department of Radiological Technology
放射線部

Associate Professor	Hiroyuki Akai, M.D., D.M.Sc.	准教授	博士(医学)	赤井宏行
Head Radiologic Technologist	Kenji Ino, RT	放射線技師長		井野賢司

The Department of Radiology undertakes radiology service at IMSUT hospital. Our expertise includes general diagnostic radiology, neuroradiology, clinical nuclear medicine, and radiation therapy. Board-certified radiologists at the Department of Radiology conduct all examinations of CT, MRI, and nuclear medicine. Radiological reports are made by the radiologists. In addition, several clinical studies are being conducted in collaboration with other departments or institutions. We also investigate the technical aspects of molecular imaging with intact small animals for its application to preclinical studies using an optical imaging system and MRI.
The Department of Radiological Technology constitutes the hospital radiology service together with the Department of Radiology. Plain radiography, dual-energy X-ray absorptiometry, and barium studies are also available at the Department of Radiological Technology, other than CT, MRI, and radioisotope examinations. More than 10,000 patients visit our department every year. Radiologic technologists at the department make an effort to provide high-quality medical images in daily practice as well as to reasonably reduce radiation exposure of a patient during the examination.

Effects of negativity bias on amygdala and anterior cingulate cortex activity in short and long emotional stimulation paradigms

Yoshida N, Kotani Y[1], Ohgami Y[1], Kunimatsu A[2], Inoue Y[3], Kiryu S[4], Okada Y[5]
[1]Institute for Liberal Arts, Tokyo Institute of Technology, [2]Department of Radiology, International University of Health and Welfare Mita Hospital, [3]Department of Diagnostic Radiology, Kitasato University, [4]Department of Radiology, International University of Health and Welfare Narita Hospital, [5]Department of Radiological Technology, Graduate School of Health Science, Suzuka University of

Medical Science

In assessing the amygdala and anterior cingulate cortex (ACC), which is reported to be evaluation targets of major depressive disorder, a short and simple stimulation paradigm could be preferable to reduce the burden on patients. However, negativity bias, which is the phenomenon by which negative stimuli are processed noticeably faster than positive stimuli, might affect the activation of these regions in the short and simple stimulation paradigm. In the present study, we aimed to assess the effects of negativity bias on the amygdala and ACC as a result of manipulating the stimulation paradigm (short-simple vs. long-complex conditions) on presenting pleasant and unpleasant pictures. As a result, image analyses showed that the amygdala was activated during unpleasant picture presentation, regardless of the task length, but no activation was observed during pleasant picture presentation under the short-simple condition. The ACC was deactivated in both the short-simple and long-complex conditions. Region of interest analyses showed that the effect of negativity bias was prominent for the amygdala in the short-simple condition and the ACC in the long-complex condition. In conclusion, the effects of negativity bias depend on neural regions, including the amygdala and ACC, and therefore, we should consider these effects while designing stimulation paradigms.

Effects of Gadolinium Deposition in the Brain on Motor or Behavioral Function: A Mouse Model

Akai H, Miyagawa K[6], Takahashi K[6], Mochida-Saito A[6], Kurokawa K[6], Takeda H[7], Tsuji M[6], Sugawara H, Yasaka K[8], Kunimatsu A, Inoue Y, Abe O[8], Ohtomo K[9], Kiryu S
[6]Department of Pharmacology, School of Pharmacy, International University of Health and Welfare, [7]Department of Pharmacology, School of Pharmacy at Fukuoka, International University of Health and Welfare, [8]Department of Radiology, Graduate School of Medicine, University of Tokyo, [9]International University of Health and Welfare

Recent studies showing gadolinium deposition in multiple organs have raised concerns about the safety of gadolinium-based contrast agents (GBCAs). In the present study, we explored whether gadolinium deposition in brain structures will cause any motor or behavioral alterations. Groups of 17 female BALB/c mice were each repeatedly injected with phosphate-buffered saline (control group), a macrocyclic GBCA, or a linear GBCA for 8 weeks (5 mmol per kilogram of bodyweight per week for GBCAs). Brain MRI studies were performed every other week to observe the signal intensity change caused by the gadolinium deposition. After the injection period, rotarod performance test, open field test, elevated plus-maze test, light-dark anxiety test, locomotor activity assessment test, passive avoidance memory test, Y-maze test, and forced swimming test were performed to assess the locomotor abilities, anxiety level, and memory. As a result, gadolinium deposition in the bilateral deep cerebellar nuclei was confirmed with MRI only in mice injected with a linear GBCA. Behavioral analyses showed that locomotor abilities, anxiety level, and long-term or short-term memory were not different in mice injected with linear or macrocyclic In conclusion, no motor or behavioral alterations were observed in mice with brain gadolinium deposition. Also, the findings support the safety of macrocyclic gadolinium-based contrast agents.

Tumor size in patients with severe pulmonary emphysema might be underestimated on preoperative CT.

Sugawara H, Watanabe H[10], Kunimatsu A, Abe O, Yatabe Y[11], Watanabe SI[12], Kusumoto M[10]
[10]Department of Diagnostic Radiology, National Cancer Center Hospital, [11]Department of Diagnostic Pathology, National Cancer Center Hospital, [12]Department of Thoracic Surgery, National Cancer Center Hospital

We evaluated the effect of emphysema on tumor diameter measured on preoperative computed tomography (CT) images versus pathological specimens. Patients who underwent primary lung cancer surgery: 55 patients (57 tumors) with severe emphysema and 57 patients (57 tumors) without emphysema were investigated. The tumor diameters measured in the postoperative pathological specimens were compared with those measured on the axial CT images and on multiplanar reconstruction (MPR) CT images by two independent radiologists; a subgroup analysis according to tumor size was also performed. In the emphysema group, the mean axial CT diameter was significantly smaller than the mean pathological diameter ($p = 0.025/0.001$ for reader 1/2), whereas in the non-emphysema group, the mean axial CT diameter was not significantly different from the pathological one for both readers. The difference between CT axial diameter and pathological diameter (= CT diameter − pathological diameter) was significantly smaller (i.e., had a stronger tendency toward underestimation on radiological measurements) in the emphysema group compared with the non-emphysema group ($p = 0.014/0.008$ for reader 1/2), and the difference was significantly smaller in tumors sized > 30 mm than tumors sized ≤ 20 mm in both groups. Tumor size is significantly smaller on preoperative CT in patients with severe emphysema compared to patients without emphysema, especially in the case of large tumors. We elucidated that MPR measurement using the widest of three dimensions should be used to select T-stage for patients with severe emphysema.

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IMSUT Hospital

Department of Palliative Medicine and Advanced Clinical Oncology

先端緩和医療科

Project Senior Assistant Professor	Yasuki Hijikata, M.D., Ph.D.		特任講師	博士(医学)	土方康基
Assistant Professor	Tetsuya Ito, M.D., Ph.D.		助教	博士(医学)	伊藤哲也
Project Assistant Professor	Akira Kanamoto, M.D., Ph.D.		特任助教	博士(医学)	金本彰

We explore and provide personalized cancer treatment based on genome analysis, in addition to established standard therapy. Our goal is set also to improve patients' quality of life by controlling symptoms related to the disease and treatment. We will perform a multidisciplinary approach to medical care based on the two specialized perspectives of cancer treatment and palliative medicine.

1. Clinical sequencing in patients with refractory advanced cancers.

Yasuki Hijikata[1], Akira Kanamoto[1], Tetsuya Ito[1], Masahiro Ikeda[1], Noriko Fujiwara[1], Kiyoshi Yamaguchi[2], Tsuneo Ikenoue[2], Seiya Imoto[3], Yasuo Matsubara[4], Kazuaki Yokoyama[5], Hiroshi Yotsuyanagi[4], Yoichi Furukawa[2]

[1] Dept. of Palliative Medicine / Advanced Clinical Oncology, IMSUT Hosp.
[2] Div. of Clinical Genome Research, IMSUT
[3] Div. of Health Medical Intelligence Human Genome Center, IMSUT
[4] Dept. of General Medicine, IMSUT Hosp.
[5] Dept. of Hematology/Oncology, IMSUT Hosp.

Metastatic cancer is a major cause of death and is associated with poor treatment efficacy. A better understanding of the advanced cancers is required to help adapt personalized treatments. Next-generation sequencing (NGS)-based genomic testing for cancer is becoming more widespread as a clinical tool for accurate diagnosis and proper treatment in clinical oncology. However, using various NGS techniques to guide cancer therapy has created challenges in analyzing large volumes of genomic data and reporting results to patients and caregivers. To resolve this, we organized a clinical sequencing team called the molecular tumor board (MTB). Clinical sequencing is associated with several potential challenges in analysis, interpretation, and drug development for refractory cancers. Briefly, after obtaining informed consent, whole-exome sequencing and/or RNA sequencing were performed on tumor, for comparisons with normal tissue, followed by analysis our hospital curators. MTB chose actionable drugs based on artificial intelligence and our database. The chosen drugs are administered to patients with advanced cancers refractory to standard treatment in our clinical study. We are currently evaluating the results of clinical study.

2. Palliative medicine to improve QOL of patients with life-threatening illness and their families.

Tetsuya Ito[1], Noriko Fujiwara[1], Akira Kanamoto[1], Masahiro Ikeda[1], Aya Watanabe[1], Tomoe Honda[1], Yasuki Hijikata[1]

[1] **Dept. of Palliative Medicine / Advanced Clinical Oncology, IMSUT Hosp.**

Patients with life-threatening illness including cancer and their families are facing challenges, that interfere with their quality of life.

Regardless of the stage of the disease, we aim to address problems of patients and families, whether physical, psychological, social or spiritual, and eventually improve their quality of life under multidisciplinary collaboration.

At the same time, we will conduct research activities to build evidence on palliative medicine and disseminate new findings.

A total of 93 inpatients were treated from January to December 2021.

Malignancy 81: Esophagus 1, Gastric 15, Pancreas 18, Colorectal 21, Lung 4, Breast 2, Hematological 2, Gynecologial 2, Melanoma 3, Others 13.

Others 12

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Murahashi M, Tsuruta T, Yamada K, Hijikata Y, Ogata H, Kishimoto J, Yoshimura S, Hikichi T, Nakanishi Y, Tani K. Clinical Trial of a Cancer Vaccine Targeting VEGF and KIF20A in Advanced Biliary Tract Cancer. Anticancer Res. 41(3):1485-1496, 2021.

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IMSUT Hospital

Department of Diagnostic Pathology
病理診断科
Department of Pathology
病理部

Associate Professor	Yasunori Ota, M.D., Ph.D.	准教授	博士(医学)	大 田 泰 徳
Project Assistant Professor	Tamami Denda, Ph.D.	特任助教	博士(保健学)	傳 田 珠 美

Our mission

1. *We provide an accurate and high-quality pathological diagnosis to the patient in this research hospital, The Institute of Medical Science, The University of Tokyo.*
2. *Make diagnosis by morphological approach using microscope to the laboratory materials.*

Overview
We study about the hematological malignancy and transplantation pathology. We emphasize many clinical cases and write case reports about human diseases.
We also perform pathological and cytological diagnosis of many specimens submitted by various departments.

1. Effusion-based lymphoma (EBL) in Japan.

Effusion-based lymphoma is found in pleura or ascites and usually lack of evidence for nodular lesion. Conventional findings about EBL are bad clinical course and many patients are infected by HIV. However, some of Japanese patients were not infected HIV and good clinical course. We reported some case reports about EBL in Japan and are going to promote multi-institutional joint research in Japan. We therefore conducted a retrospective study of 95 patients with EBL, regardless of HHV8 status, in Japan. Of 69 patients with EBL tested for HHV8, a total of 64 were negative. The median age of patients with primary HHV8-negative EBL at diagnosis was 77 years (range, 57-98 years); all 58 tested patients were negative for HIV. Primary HHV8-negative EBL was most commonly diagnosed in pleural effusion (77%). Expression of at least 1 pan B-cell antigen (CD19, CD20, or CD79a) was observed in all cases. According to the Hans algorithm, 30 of the 38 evaluated patients had nongerminal center B-cell (non-GCB) tumors. Epstein-Barr virus–encoded small RNA was positive in 6 of 45 patients. In 56 of 64 HHV8-negative patients, systemic therapy was initiated within 3 months after diagnosis. Cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP) or CHOP-like regimens with or without rituximab (n = 48) were the most common primary treatments. The overall response and complete response rates were 95% and 73%, respectively. Three patients did not progress without systemic treatment for a median of 24 months. With a median 25-month follow-up, the 2-year overall survival and progression-free survival rates were 84.7% and 73.8%. Sixteen patients died; 12 were lymphoma-related deaths. Thus, most EBL cases in Japan

are HHV8-negative and affect elderly patients. The non-GCB subtype is predominant. Overall, primary HHV8-negative EBL exhibits a favorable prognosis after anthracycline-based chemotherapy.

2. Medical Activities

We have performed microscopic diagnosis of many pathological and cytological samples. We also provided immunohistochemical analysis and in situ hybridization in order to improve the diagnostic accuracy and decide the treatment.

Pathological diagnosis n = 1406
- Endoscopic samples n = 937
- Surgical resection n = 241
  - Fine-needle aspiration n = 179
  - Intraoperative diagnosis n = 21
  - Consultation n = 20
  - Other n = 8

Cytological diagnosis n = 384

Autopsy n = 1

3. Pathology Core Laboratory II

Pathology Core Laboratory II handles a large number of specimens, including mouse, cultured cells and human tissue samples collected at the IMSUT hospital. We have performed preparation of pathological specimen and pathological analysis (n = 90).

Publications

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IMSUT Hospital

Department of Gastroenterology
消化器内科

Associate Professor Yoshihiro Hirata, M.D., D.M.Sc.
Senior Assistant Professor Yasuo Matsubara, M.D., D.M.Sc.

准教授 博士(医学) 平 田 喜 裕
講 師 博士(医学) 松 原 康 朗

The department of gastroenterology was founded in 2021 to provide the specialized examination and treatment of digestive diseases for the IMSUT hospital patients in collaboration with department of surgery and department of oncology and general medicine. Multiple clinical research project is also underway with other IMSUT departments.

1. Treatment of drug-resistant *Helicobacter pylori* infection

Matsubara Y., Hirata Y.

Some patients fail to respond first- and second-line *Helicobacter pylori* (*H. pylori*) eradication therapy, but third-line eradication is not always done. Meanwhile, penicillin allergy patients do not take routine eradication medicines because insurance coverage regimens in Japan include amoxicillin. In *H. pylori* out-patient clinic of IMSUT hospital, we make correct diagnosis of infection by multiple modalities, give eradication therapy for these refractory patients, and achieve high rates of successful eradication.

2. Endoscopic examination in IMSUT Hospital

Matsubara Y., Hirata Y.

About 700 cases of upper gastrointestinal endoscopy and about 250 cases of colonic endoscopy were performed in 2021. We have diagnosed rare diseases (e.g. infectious disease, malignancy, other disease) in patients with immune dysfunction. We also participated in endoscopic health check up in Minato Ward.

3. Diagnosis and treatment of inflammatory bowel disease.

Matsubara Y., Hirata Y.

Inflammatory bowel disease is a digestive disease entity with unknown cause. Patients usually exhibit abdominal symptoms and show inflammation in gastrointestinal tract. We provide basic and up-to-date therapy (biologic and small-molecule medicines) for the patients.

Publications

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IMSUT Hospital

Department of Surgery
外科

Professor	Dai Shida, M.D., Ph.D.	教　授	博士(医学)	志　田　　大
Associate Professor	Susumu Aiko, M.D., Ph.D.	准教授	博士(医学)	愛　甲　　丞
Associate Professor	Masaru Shinozaki, M.D., Ph.D.	准教授	博士(医学)	篠　崎　　大
Senior Assistant Professor	Giichiro Tsurita, M.D., Ph.D.	講　師	博士(医学)	釣　田　義一郎
Assistant Professor	Yuka Ahiko, M.D.	助　教		阿　彦　友　佳
Assistant Professor	Taro Tanabe, M.D.	助　教		田　邊　太　郎

The mission of our department is to provide surgical treatment for various gastrointestinal diseases, such as colorectal cancers, gastric cancers, and inflammatory bowel diseases. Since the participation of Prof. Shida and Dr. Ahiko in September 2020, as well as Dr. Aiko in November 2020, we have mainly performed laparoscopic surgery (i.e., colectomy, low anterior resection, gastrectomy) instead of open surgery for these diseases. In addition, we started Robotic surgery in April, 2021. We also treat acute appendicitis and cholecystitis, and perform diagnostic and therapeutic gastrointestinal endoscopy.

1. Introduction

Professor Shida and Dr. Ahiko newly joined in September 2020. And Dr. Aiko also newly joined in November 2020. We specialize in the treatment of gastrointestinal cancers, especially surgical treatment of colorectal cancer and gastric cancer. Colorectal cancer can be completely cured by more than 70% of patients when appropriate surgery is performed, even if it is stage III cancer. As qualified surgeons (endoscopic surgical skill qualification system) of the Japan Society for Endoscopic Surgery (Dr. Shida and Dr. Aiko), we are actively performing minimally invasive surgery with less physical burden of patients. In addition, Dr. Shinozaki specializes in the treatment of inflammatory bowel diseases. All the staff will propose the most suitable treatment method according to the medical condition and will do our best to treat the patients.

2. Treatment for gastrointestinal malignancy

Colorectal cancers and gastric cancers are what we mainly treat. For rectal cancer, in order to improve the QOL (quality of life) after surgery as much as possible, we select not only autonomic nerve-sparing surgery but also anus-sparing surgery if the cancer can be sufficiently resected. For gastric cancer, we select the surgical method with policy of 'leaving the remaining stomach as much as possible', because stomach surgery limits the amount of food that patients eat after surgery which leads to weight loss and weakness. As qualified surgeons (endoscopic surgical skill qualification system) of the Japan Society for Endoscopic Surgery (Dr. Shida and Dr. Aiko), we are actively performing minimally invasive surgery, that is, laparoscopic surgery.

<January 2021 – December 2020>

Colorectal cancer / Rectal NET (neuroendocrine tumor): n = 100

- Open n = 4
- Laparoscopic n = 81
- Robotic n = 15

Gastric cancer n = 16

Gastric GIST n = 1

3. Treatment for inflammatory bowel disease

We treat inflammatory bowel diseases such as ulcerative colitis and Crohn's disease. As board certified surgeons in gastroenterology (Dr. Shia, Dr. Aiko, Dr. Tsurita), laparoscopic surgery for ulcerative colitis is also performed (mainly by Dr. Shinozaki).

4. Surgical treatment for benign diseases

We also treat a variety of benign diseases such as acute appendicitis, cholecystitis, and colonic diverticulitis.

5. Endoscopic examination and treatment

Under cooperation with Department of General Medicine (Dr. Matsubara Y. and Dr. Hirata Y.), we performed many cases of upper gastrointestinal endoscopy and colonoscopy.

6. Launch of Robotic Surgery

We started robotic rectal surgery for rectal tumors such as rectal cancer and rectal NET (neuroendocrine tumor) in April, 2021.

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IMSUT Hospital

Department of Anesthesia
麻酔科

Associate Professor	Ryo Orii, M.D., Ph.D.	准教授　博士(医学)	折　井　　亮
Assistant Professor	Miho Asahara, M.D., Ph.D.	助　教　博士(医学)	浅　原　美　保

Our clinical practice and clinical studies have been focused on (1) assessment of functional failure of anesthesia machine (2) anesthetic management in patients undergoing major surgery including joint arthroplastic surgery for hemophilia patients, variable surgical procedures for translational researches (3) assessment of reliability of cardiac output measurements (4) risk management of medical electronic devices in Research Hospital.

1. Assessment of functional failure of the internal valve applying maximum and positive end-expiratory pressure of anesthesia machine

Equipment-related complications, whatever its cause, should be prevented by checking the breathing system prior to general anesthesia. We found irregularities with some of the anesthesia machines at our department, which was related to a ventilator-related problem that recurred after application of positive end-expiratory pressure (PEEP) during general anesthesia.

The issue with the PEEP/Pmax valve, which can lead to changes in flow and pressure during mechanical ventilation, could go unnoticed because the valve is encased inside the breathing circuit, and requires disassembly for close inspection. Our findings highlight the importance of keeping the anesthetic circuit, including the internal components of the PEEP/Pmax valve, free of unexpected contamination through more thorough preventive maintenance cycles.

2. Anesthetic management for carrier hemophilia.

Hemophilia is X-linked gene disease with the activity abnormality of the coagulation factor. The hemophilia A is caused by factor VIII abnormality, and the hemophilia B is caused by factor Ⅸ abnormality. Careful hemostatic management is required in perioperative care of the hemophilic patients. It is usually recommended that we perform coagulation factor replacement therapy and hemostatic monitoring.

We experienced anesthesia management of the orthopedic surgery of patients with hemophilia B that underwent living-donor liver transplantation for cirrhosis due to the hepatitis C virus this time. We carried out hemostatic monitoring and perioperative management, but did not require coagulation factor replacement therapy. There were no complications such as postoperative bleeding and infection.

Female hemophilia patients are often not informed as carriers themselves, and there is a possibility that medical practice may be performed without recognizing them as hemophilia patients. We experienced anesthesia of female hemophilia patients and safety managed anesthesia with appropriate hemostatic management.

3. Assessment of reliability of cardiac output measurements.

Knowing a patient's cardiac output (CO) could contribute to a safe, optimized hemodynamic control during surgery. Precise CO measurements can serve

as a guide for resuscitation therapy, catecholamine use, differential diagnosis, and intervention during a hemodynamic crisis. Despite its invasiveness and intermittent nature, the thermodilution technique via a pulmonary artery catheter (PAC) remains the clinical gold standard for CO measurements. LiDCO rapid™ (LiDCO, London, UK) and FloTrac/Vigileo™ (Edwards Lifesciences, Irvine, CA) are less invasive continuous CO monitors that use arterial waveform analysis. Anesthesiologists use FloTrac/Vigileo™ in our operating room.

We found both devices tended to underestimate the caluculated CIs when the CIs were relatively high. These proportional bias produced large parcentage errors in the present study.

4. Risk management of medical electronic devices.

We ourselves engage in preventive maintenance and care of the life support machines including instruments for mechanical ventilation. We also supervise physicians during clinical usage of these instruments.

Publications

Unexpected deposits in the anesthetic circuit: a possible cause of PEEP/Pmax valve malfunction
Ikeda, T., Orii, R., Iwakiri, M., Uchida, K., Yamada, Y.
Journal of Clinical Monitoring and Computing 2021, 35(4), P.943-948

IMSUT Hospital

Department of Joint Surgery
関節外科

Senior Assistant Professor	Hideyuki Takedani, M.D., D.M.Sc.	講　師　博士(医学)	竹　谷　英　之
Assistant Professor	Kumiko Ono, M.D., D.M.Sc.	助　教　博士(医学)	大　野　久美子

Department of Joint Surgery was established in 2006. Our clinical mission is evaluation and treatment of hemophilic arthropathy. In Japan, many hospitals are able to control bleeding for haemophilia by concentrates, however there are few hospitals focus on surgical treatments except us. Many haemophilia patients come to our department from all over Japan. We evaluate their joint condition and function roentgenographically and physiotherapeutically and decide indication of surgical treatment. Many of patients will be performed joint arthroplasties and arthroscopic synovectomy to improve their quality of life. We researched how to control bleeding adequately during perioperative period as well.

As basic mission, we started the research for pathogenesis of hemophilic arthropathy, collaborated with the department of orthopedic surgery, the University of Tokyo. The aim of this research is to develop mesenchymal stem cell therapy for hemophilic arthropathy.

From 2006 to 2020, more than 253 surgical treatments for hemophilia included other coagulation diseases such as deficiency factor VII or Von Willebrand disease. Some of them have the deficiency factor antibody as well.

Publication 2020

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IMSUT Hospital

Department of Surgical Neuro-Oncology
脳腫瘍外科

Professor	Tomoki Todo, M.D., Ph.D.	教　授	医学博士	藤　堂　具　紀
Project Associate Professor	Minoru Tanaka, M.D., Ph.D.	特任准教授	医学博士	田　中　　実
Assistant Professor	Hirotaka Ito, M.D., Ph.D.	助　教	医学博士	伊　藤　博　崇
Assistant Professor(Thoracic surgeon)	Yoshinori Sakata, M.D., Ph.D.	助　教	博士(医学)(呼吸器外科医)	坂　田　義　詞

All kinds of brain tumors, especially malignant glioma, are treated at our department. Malignant glioma is incurable by standard therapy alone, therefore refined, personalized treatment regimens utilizing non-standard radiation therapy and chemotherapy are considered. In addition, G47Δ, the first oncolytic virus therapy drug for malignant glioma in the world, developed by this department, is commercially available and used for treatment since November 2021. Based on scientific evidence and findings from basic research, we conduct advanced medical practices in addition to standard therapy.

Introduction

Department of Surgical Neuro-Oncology was established in 2011. Our department started treating out-patients in October 2011 and in-patients in April 2012. Our department focuses on malignant tumors of the brain, such as gliomas or metastatic brain tumors. Glioblastoma is one of the most aggressive and malignant cancers of the central nervous system. The standard upfront treatment includes resection to remove as much of the tumor as possible while preserving function, followed by radiation of 60Gy and temozolomide. Established good prognostic factors are limited but include young age, high Karnofsky Performance Status (KPS), high mini-mental status examination score, O^6-methylguanine methyltransferase promoter methylation, and resection of > 98% of the tumor. Nevertheless, glioblastoma is refractory to conventional therapies and has a poor prognosis with a 5-year survival rate of less than 5%. Therefore, we should consider refined and personalized treatment approaches for selected patients: high dose radiation therapy of 80Gy for newly diagnosed glioblastoma or extended field stereotactic radiosurgery for recurrent gliomas. We also conduct translational research based on scientific evidence. We are developing recombinant herpes simplex virus type I (HSV-1), which has genetic modifications in the viral genome so that the viruses replicate selectively in cancer cells while eliciting an immune response against tumor-associated proteins. Clinical trials using a third-generation, triple-mutated oncolytic herpes simplex virus type 1 (HSV-1), G47Δ, were performed in patients with glioblastoma from 2015 to 2020 and malignant pleural mesothelioma from 2018 to 2021. The clinical trial is ongoing in patients with olfactory neuroblastoma. We also started a new investigator-initiated clinical trial using T-hIL12 for malignant melanoma jointly with Shinshu University since January 2020.

A phase II clinical trial of a replication-competent, HSV-1, G47Δ in patients with glioblastoma

Genetically engineered, conditionally replicating HSV-1is promising therapeutic agents for solid carcinomas. We developed G47Δ by introducing an additional genetic mutation to a second generation, double-mutated oncolytic HSV-1, G207, used in the phase I clinical trial for glioblastoma in the United States in 1998. We conducted a phase II clinical trial of G47Δ in

patients with glioblastoma since December 2014 to June 2020. The main inclusion criteria were a recurrent or residual glioblastoma with a single lesion (≥1cm) after initial radiation therapy concomitant with temozolomide chemotherapy, age 18 or older, life expectancy of at least 3 months, a performance-status according to Karnofsky Performance Scale of ≥ 60% and adequate organ function. The eligible patients received repeated stereotactic injections with G47Δ every 4 weeks, 6 injections being the maximum total. The efficacy of G47Δ evaluates using a one-year survival rate as the primary endpoint. The planned interim analysis showed significant efficacy with limited side effects of G47Δ, so the trial was terminated early. In the final analysis, the 1-year survival rate after initiation of G47Δ treatment (primary endpoint) was 84%, and the most common side effect of G47Δ was fever followed by vomiting, nausea, lymphopenia, and leukopenia. A new drug application (NDA) for G47Δ for malignant glioma has been submitted to the Ministry of Health, Labour and Welfare in December 2020. In June 2021, G47Δ was approved as the world's first oncolytic virus drug for malignant glioma. The oncolytic virus therapy using G47Δ for the patient with malignant gliomas started at this department in November 2021 upon commercial distribution.

A clinical study of G47Δ in patients with progressive olfactory neuroblastoma

Olfactory neuroblastoma is an uncommon malignant neuroectodermal tumor, which is thought to originate from the olfactory membrane of the sinonasal tract. Patients should receive aggressive treatment with combined treatment such as surgery, radiation therapy, and chemotherapy because there is no effective treatment once it recurs: An aggressive en bloc resection, with combined radiation therapy was recommended. We have been conducting a phase I clinical trial of G47Δ in patients with progressive olfactory neuroblastoma since August 2013. The key inclusion criteria are histologically confirmed recurrent olfactory neuroblastoma despite previous or ongoing radiation therapy, age 18 or older, a measurable tumor lesion (≥1cm) on gadolinium-enhanced T1-MRI of the brain, life expectancy of at least 3 months, a performance-status of 0-2 and adequate organ function. In this protocol patients with advanced disease (eg, Kadish stage C) are covered. G47Δ will be repeatedly inoculated to the residual tumor innasal cavity every 4 weeks until tumor progression or excessive toxicity occurred. The primary endpoint is safety, and the secondary endpoints include efficacy analysis.

A clinical study of G47Δ in patients with progressive malignant pleural mesothelioma

Malignant pleural mesothelioma is a rare asbestos-induced malignancy with an estimated incidence of approximately 2,000 new cases diagnosed in Japan. Worldwide, nearly 80% of mesothelioma deaths occur in ten countries, with Japan, the United Kingdom, and the United States being in the top three. It is expected to continue to increase over the next several decades. Median survival ranges from 9 to 18 months and correlates with stages. Radiotherapy can be used for different indications in mesothelioma: palliation, as a preventive treatment, and as part of multimodality treatment. Combination doublet chemotherapy of cisplatin, with either pemetrexed or raltitrexed, has shown a more prolonged survival compared with cisplatin alone in randomized phase III trials. Carboplatin is an acceptable alternative to cisplatin and may be better tolerated in the elderly population. We conducted a phase I clinical trial of G47Δ for malignant pleural mesothelioma from 2018 to 2021. The key inclusion criteria were histologically confirmed malignant pleural mesothelioma that was inoperable, recurrent or progressive, no prior thoracotomy or thoracoscopic surgery, except for biopsy, age 20 or older, presence of one or more evaluable lesions on contrast-enhanced CT scan, interval of 4 weeks or more from prior chemotherapy if it was given, life expectancy of at least 3 months, a performance-status of 0-1 and sufficient major organ functions. In this protocol history of chemotherapy or radiotherapy was irrelative. A fixed dose of G47Δ was administered into the pleural cavity every 4 weeks, maximum 6 times. The primary endpoint was safety, and the secondary endpoints included efficacy analysis. We completed the enrollment and confirmed the safety of repeated intrapleural administration with G47Δ.

A phase 1/2 clinical trial of a recombinant herpes simplex type 1 with human IL-12 expression, T-hIL12, in patients with malignant melanoma

Malignant melanoma is a tumor produced by the malignant transformation of melanocytes. Melanocytes are derived from the neural crest; consequently, melanomas, although they usually occur on the skin, can arise in other locations where neural crest cells migrate, such as the gastrointestinal tract and brain. The 5-year relative survival rate for patients with stage 0 melanoma is 97%, compared with about 10% for those with stage IV disease. We started a phase 1/2 clinical trial of T-hIL12 in patients with malignant melanoma since January 2020 jointly with Shinshu University. T-hIL12 is a G47Δ-based recombinant herpes simplex type I with IL-12 expression. This IL-12-mediated antitumor immunity could be T-cell-mediated. The main inclusion criteria in phase 1 are 1) histologically confirmed malignant melanoma with stage 3 or 4, 2) patients who have at least one metastatic skin lesion with 10 mm or larger (the longest diameter), or at least one metastatic lymph node with 15 mm or larger (the shortest axis), 3) patients

who were administered with anti-PD-1 antibody, or targeted molecular drugs, 4) the size and distribution of all the metastatic lesions are recognized with clinical findings including imaging studies (CT, MRI), 5) age >=20 years, 6) more than 30 days have passed from the previous treatment, 7) Eastern Cooperative Oncology Group (ECOG) performance Status (PS) of 0-2, 8) patients without severe disorders (severe myelosuppression, liver dysfunction, chronic renal dysfunction), whereas in phase 2 they are eight items, which are defined in the same way as in the phase 1 except for 3) of phase1. The 3rd inclusion criterion of phase2 is 3) patients who have not been administered with anti-PD-1 antibody or targeted molecular drugs. T-hIL12 will be administered into the tumor of skin or lymph node metastases in patients with advanced stage of malignant melanoma. The assigned dose will be repeatedly inoculated into the metastases 2 or 4 times, with an interval of 14 (14 -28) days. The primary endpoint in phase 1 is safety, and in phase 2 a response rate (RECIST 1.1). The phase 2 part of this trial is ongoing.

Routine activities

Patients with brain tumors are treated by four neurosurgeons. A total of 25 operation were carried out in 2021 including 20 gliomas and 5 malignant pleural mesotheliomas. Fifteen cases of oncolytic virus therapy were performed. Standard craniotomies and image guided stereotactic biopsies of deep seated lesions, as well as high-tech brain tumor resections have been performed. The high-tech equipment regularly used in brain tumor resection surgeries includes an operative microscope, a 3-D neuro-navigation system, intraoperative motor evoked potential (MEP and SEP) recording, intraoperative ultrasonography and an ultrasonic surgical aspirator.

Patients with newly diagnosed malignant glioma have been treated with high dose or standard dose radiation therapy and concomitant chemotherapy. Temozolomide was administered to glioma patients during radiation therapy followed by a maintenance therapy every 28 days for as long as possible. The overall survival of patients with glioblastoma was 30.3 months (95% confidence interval, 24.5-36.1 months. The five-year overall survival rate was 26.5%.

Recurrent malignant glioma patients are treated with innovative non-standard therapies whenever possible. Recurrent glioma patients who have small lesions, receive extended field stereotactic radiosurgery. To enhance the efficacy of stereotactic radiosurgery (SRS), the irradiation field is enlarged to include as many tumor cells invasive to the surrounding tissue as possible. We demonstrated 93% local control in patients who received 20 Gy to a 0.5-1.0 cm extended field SRS compared to 47% of patients who were treated with 20 Gy to the gadolinium-enhancing margin only.

Treatment of primary central nervous system lymphoma

Primary central nervous system lymphoma patients will first undergo biopsy for pathological diagnosis. In addition to the standard therapy regimen using high-dose methotrexate followed by radiotherapy, an advanced treatment regimen utilizing rituximab, methotrexate, procarbazine, and vincristine (R-MPV) therapy followed by consolidation whole-brain radiation therapy has been used as a treatment option.

IMSUT Hospital

Department of Urology
泌尿器科

Professor	Haruki Kume, M.D., Ph.D.	教　授	博士(医学)	久　米　春　喜
Project Senior Assistant Professor	Sayuri Takahashi, M.D., Ph.D.	特任講師	博士(医学)	高　橋　さゆり
Project Assistant Professor	Naoki Kimura, M.D., Ph.D.	特任助教	博士(医学)	木　村　直　樹
Project Assistant Professor	Daiji Watanabe, M.D., Ph.D.	特任助教	博士(医学)	渡　邉　大　仁

Our mission is to activate the function of ISMUT hospital by introducing advanced medical treatments such as robotic surgery. We successfully performed 35 cases of robotic surgery and recruited new patients, which resulted in increase of the revenue. Further, we have been engaged in basic research on castration resistant prostate cancer to discover novel drugs by the method of molecular and cell biology.

1. Introduction of advanced surgical operations

Since we established the department of Urology in IMSUT Hospital in July,2020, the number of patients has been increased by introduced from urological clinics, hospitals, and other departments of our hospital. Totally 1,145 patients visited our department in 2021 for the purpose of thorough examinations for diagnosis or surgical treatments.

We totally performed 256 surgical operations in 2021: 28 cases of Robotic-assisted prostatectomy, two Robotic-assisted partial nephrectomy, two Laparoscopic nephrectomy, two Laparoscopic nephroureterectomy, one Radical cystectomy with ileal conduit urinary diversion, six open surgery, 45 Trans-urethral resection of bladder tumor, four Trans-urethral resection of prostate, 14 Trans-urethral lithotripsy, 22 Ureteroscopy, 35 Retrograde pyelogram, 26 Ureteral stenting, one Cystoscopy with hydrodistention, and three Botox injection for overactive bladder.

The Ministry of Health and Welfare regulates that 20 Robotic-assisted prostatectomies must be performed for one year to be paid by the health insurance. We met the standard.

We successfully expanded the medical network with outside facilities to gain new patients by introduction.

2. Basic research

Molecular targeted therapy by olaparib has improved survival for castration-resistant prostate cancer (CRPC) patients but effects on only cancer with genetic mutations. Further drug discovery targeting epigenetic modulators is required. We previously identified ESS2 as a novel transcriptional coregulator, but its function remains still unclear in cancer cells. We established ESS2 knockdown in PC3 cells (PC3-shESS2) and found that PC3-shESS2 cells dramatically inhibited proliferation in xenograft nude mice. Microarray analysis revealed that ESS2 regulates mRNA levels of chromatin remodeling factor CHD1-related genes, and other cancer-related genes such as PPAR-g, WNT5A and TGF-b, some of genes showed expressional correlation with ESS2 in prostate cancer patients. In addition, ESS2 knockdown reduced the recruitment of NFkB/CHD1 and levels of histone H3K36me3 on the target genes (TNF and CCL2) promoters. Interestingly, tamoxifen-inducible Ess2 knockout (iEss2KO) mice delayed prostate development with hypoplasia and disrupted the structure of luminal cells in ventral prostate. Our findings identify

ESS2 as a potentially novel epigenetic therapeutic target for CRPC.

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IMSUT Hospital

Department of Medical Informatics
医療情報部

Associate Professor	Hiroyuki Akai, M.D., D.M.Sc.	准教授	博士(医学)	赤井宏行
Senior Assistant Professor	Toshihiro Furuta, M.D., D.M.Sc.	講師	博士(医学)	古田寿宏
Assistant Professor	Masaru Kamitani, M.D.	助教		神谷勝

Department of Medical Informatics is engaged in the management of hospital information systems, including infrastructure for the system and the electric medical records, at the Institute of Medical Science (IMSUT) Hospital. Hospital information system enables medical staff to securely provide patient care and helps to conduct clinical research. The current hospital information system has been renewed for better patient care since 2017.
We also devote ourselves to the development and improvement of infrastructure for a regional community-based medical cooperation network between IMSUT hospital and other healthcare providers.

1. Management and operation of the hospital information system and network

Hiroyuki Akai, Toshihiro Furuta, Masaru Kamitani

We offer services related to the hospital information system of the IMSUT hospital. We work together with the IT service room of IMSUT, and the Information Technology Center of the University of Tokyo. We are obliged to maintain the hospital information service and the network system for better medical care, ensuring that patient medical records are saved in a standard format and are easily transferrable to other healthcare providers.
Our missions are as follows:

- Supervision, development, operation, and management of the hospital information system
- Education on the hospital information system to the medical staff
- Development and management of the network infrastructure for securely dealing with patient personal information and clinical records
- Day-to-day management and operation of the hospital information system and network
- General work concerning the operation of the hospital information system and network

2. IT support to a community-based healthcare provider network

Hiroyuki Akai, Toshihiro Furuta, Masaru Kamitani

"Community-based integrated care systems" is a keyword for the Japanese healthcare system in this decade. IMSUT hospital belongs to its community-based healthcare provider network and we continuously improve infrastructure for cooperation in the network.

Our hospital information system has been renewed since 2017. We hope that the latest electronic healthcare record system will help to refer patients from hospital to clinic and from clinic to hospital in the network.

IMSUT Hospital

Department of Cell Processing and Transfusion

セルプロセッシング・輸血部

Clinical Professor	Tokiko Nagamura-Inoue, M.D., Ph.D.		病院教授	博士(医学)	長 村 登紀子
Assistant Professor	Kazuaki Yokoyama, M.D., Ph.D.		助 教	博士(医学)	横 山 和 明
Assistant Professor	Toyotaka Kawamata, M.D., Ph.D.		助 教	博士(医学)	川 俣 豊 隆

Our department was established in 1990 to manage transfusion medicine and cell processing for hematopoietic stem-cell transplantation. In addition to transfusion-related works, our department has supported translational studies and managed the IMSUT-Cell Resource Center (IMSUT-CRC), established in 1997. Our recent projects include the Research Cord Blood Bank (RCBB); the National BioResource Project (NBRP) supported by the Ministry of Education, Culture, Sports, Science and Technology; and umbilical cord blood and umbilical cord–derived mesenchymal stromal cell (UC-MSC) banking for clinical use, supported by the Japan Agency for Medical Research and Development (Ministry of Health, Labour and Welfare). We have been studying the immunological effects of UC-MSC administration in an investigator-initiated clinical trial for treatment-resistant severe acute graft-versus-host disease since 2018 to 2020. We have been also exploring new applications of UC-MSCs in treating acute cerebral injury, hemophagocytic syndrome, and radiation injury.

1. Transfusion medicine and related tests

Abe Y, Ogami K, Iwasawa N, Yokoyama K, Kawamata T, Nagamura-Inoue T

Our department controls and supports transfusion medicine through blood typing, irregular antibody testing, and cross-matching tests on blood transfusion products including concentrated red blood cells, platelets, and frozen plasma. The blood type of some patients with hematopoietic disorders and post–stem cell transplantation is undetectable. In these cases, we perform blood typing tests with particular care, as the blood type of the patient transitions to the donor type.

2. Apheresis for peripheral blood stem-cell mobilization and collection, CAR-T, and dendritic cell therapy

Nagamura-Inoue T, Ogami K, Takahashi A, Kawamata T, Yokoyama K

For autologous peripheral blood stem cell transplantation, we perform apheresis for patients with myeloma and malignant lymphoma after mobilization by granulocyte colony-stimulating factor with or without the CXCR-4 inhibitor, Plerixafor. We evaluate the efficacy of mobilization by testing HPC- and CD34-positive cells in the peripheral blood on the day of apheresis and processing the products. We perform mobilization and apheresis for patients from the IMSUT hospital upon request. We also perform aphere-

sis to obtain lymphocytes (CD3-positive cells) for the construction of CAR-T cells and monocytes for dendritic cell therapy.

3. Therapeutic application of umbilical cord–derived mesenchymal stromal cells to severe acute graft-versus-host disease (aGVHD) and analysis of immunological influence

Nagamura-Inoue T, Takahashi A, Hori A, Miharu Y, Yamamoto Y, Nagamura F, Konuma T, Kato S, Yokoyama K, Nanya Y

We investigated the immunological influence, safety, and efficacy of allogeneic umbilical cord–derived mesenchymal stromal cells (IMSUT-CORD)—processed in a serum-free medium including a cryoprotectant—in treating steroid-resistant acute graft-versus-host disease (aGVHD). In a phase I dose-escalation clinical trial, cryopreserved IMSUT-CORD was thawed and infused intravenously twice per week over two cycles. Patients with no adverse effects and a partial (PR) or mixed response (MR) underwent up to two additional cycles. Four patients received a dose equivalent to 1×10^6 cells/kg, while three received a 2×10^6/kg dose. No severe adverse events were observed. At 16 weeks after the initial IMSUT-CORD infusion, one patient showed no response, one showed MR, two showed PR, and three showed a complete response (CR). The overall response was 71.4% (90% CI: 34.1–94.7%), while the overall survival was 85.7% (90% CI: 62.2–100%). The continuous CR/PR rate was 100% for more than 28 days after reaching CR/PR, while the survival rate was 85.7% on day 100 (90% CI: 62.2–100). The number of natural killer cells increased significantly and correlated with the treatment response, whereas IL-12, IL-17, and IL-33 levels decreased and did not correlate with treatment response. CCL11 levels increased and correlated with the treatment response. IMSUT-CORD can be used in patients with steroid-resistant aGVHD. The trial was registered at https://www.umin.ac.jp/ctr as #UMIN000032819.

4. Therapeutic application of UC-MSCs to acute brain injury

Sei K, Yamamoto Y, Mukai T, Takahashi A, Nagamura-Inoue T

In a previous study, we established a neonatal intraventricular hemorrhage (IVH, a type of neonatal brain injury) mouse model and found that the intravenous injection of UC-MSCs improved behavioral outcomes in IVH by restoring periventricular reactive gliosis, hypomyelination, and periventricular apoptosis in vivo. Based on the efficacy of proof of concept using UC-MSCs for cerebral palsy, a clinical trial (Phase I/II) was initiated in 2021 to treat cerebral palsy. In recent research, we investigated the role of microglia in acute brain injury and the improvement facilitated by UC-MSCs.

5. The Research Cord Blood Cell Resource / National BioResource Project (NBRP)

Izawa Y, Miharu Y, Yamamoto Y, Takahashi A, Nagaya N, Nagamura-Inoue T

The Research Cord Blood bank / resource was established in 2004 and supported by the Ministry of Education, Culture, Sports, Science and Technology for the development of regenerative medicine, immunological cell therapy, infection research, modified gene cell therapy, and drug discovery. Since July 2012, this project has been incorporated into the National BioResource Project (NBRP). The research umbilical cord blood (CB) bank provides processed and cryopreserved CB units (nucleated cells, mononuclear cells, and CD34 + cells) to researchers worldwide via the RIKEN Bioresource Center. The website is at http://www.nbrp.jp/.

6. Institute of Medical Science, University of Tokyo, Cell Resource Center (IMSUT-CRC)

Takahashi A, Miharu Y, Hori A, Yamamoto Y, Nagamura-Inoue T

To promote cell therapy in translational research, IMSUT-CRC was established in 1997 (originally called the Room for Clinical Cellular Technology, or RCCT). To date, the following projects have been implemented: 1) CB cell processing for banking in the manner of the Tokyo Cord Blood Bank (1997–2008), 2) research cord blood bank (2004–), 3) dendritic cell therapies (1998–2001), 4) regenerative therapy of alveolar bone derived from bone marrow mesenchymal cells (2005–2011), 5) gene therapy for renal cancer (1998), 6) CB and UC-MSC banking (IMSUT-CORD; 2012–), 6) aAVC-WT1 cell therapy (2017–), and (7) dendritic cell (DC) therapy using DCs pulsed with neoantigen (2020–).

Visit our website: http: //www.ims.u-tokyo.ac.jp/dcpt/english/

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IMSUT Hospital

Surgical Center
手術部

Professor	Tomoki Todo, M.D., Ph.D.	教　授	博士（医学）	藤　堂　具　紀
Project Associate Professor	Minoru Tanaka, M.D., Ph.D.	特任准教授	博士（医学）	田　中　　実

IMSUT hospital provides seamless support for translational research. Our mission is the management and operation of the surgical center to achieve a safe and organized environment where surgical procedures can be performed in high quality. A da Vinci surgical system (da Vinci Xi), a robotic technology that allows surgeons to perform minimally invasive procedures, was introduced in November 2020. Robot-assisted Radical Prostatectomies (RARP) for prostate cancer and robotic rectal surgery for tumors including rectal cancer and GIST are performed.

Introduction

IMSUT hospital provides seamless support for translational research. The aim is to apply knowledge gained from basic science to clinical and community health-care settings. Our mission is the management and operation of the surgical center to achieve a safe and organized environment where surgical procedures can be performed in high quality. Our activities include the management of clean areas, the establishment of protocols for infection control, maintenance of equipment such as astral lamps, surgical microscopes and fiberscopes, and organizing of daily and weekly operations. A da Vinci surgical system (da Vinci Xi), a robotic technology that allows surgeons to perform minimally invasive procedures, was introduced in November 2020, and Robot-assisted Radical Prostatectomies (RARP) for prostate cancer started. Department of surgery initiated Robotic rectal surgery for tumors including rectal cancer and GIST in 2021. Medical engineer staffs increased accordingly, and a ME Division was newly established in the Surgical Center. Three of four maintained at a NASA class 1,000 clean level and specifically designed for neurosurgery and joint surgery. For prompt and sustained supply of sterilized materials, we keep the surgical tools for each department in sets of designated purposes.

Equipment in the surgical center

The center is equipped with C-arm x-ray TV systems, surgical microscopes, ultrasonic aspirators, image guided navigation systems, intraoperative ultrasound imaging systems, intraoperative nerve simulation monitoring systems, etc. The endoscopic procedure room is located separately but adjacent to the surgical center.

TV monitoring system

Each operating room is equipped with a TV camera, so that the rooms can be monitored in the control center as well as by pad devices carried by managing anesthesiologists.

Induction of electronic ordering system

We are accommodating an electronic ordering system for the surgical center that allows a real time ordering by clinical departments and a computerized management of operation schedules.

Facts in the fiscal year 2021

Total number of operations	454
Planned operations	449

Emergency operations	5	Epidural	0
General anesthesia	281	Local	85
Spinal	88	Others	0

IMSUT Hospital

Department of Laboratory Medicine
検査部

Clinical Professor	Tokiko Nagamura-Inoue, M.D., Ph.D.		部長／病院教授	博士(医学)	長 村 登紀子
Assistant Professor	Tomohiro Ishigaki, M.D., Ph.D.		副部長／助教	博士(医学)	石 垣 知 寛
Project Senior Assistant Professor	Koichi Kimura, M.D., Ph.D.		特任講師	博士(医学)	木 村 公 一
Chief Technologist	Hironori Shimosaka		技師長	臨床検査技師	下 坂 浩 則

The department of laboratory medicine consists of seven divisions: clinical hematology, biochemistry/serology, microscopy, pathology, microbiology, physiology, and TR verification laboratory.
Clinical laboratory tests are necessary for all clinical practice steps including diagnosis of diseases, evaluation of stages, determination of treatments, and assessment after therapy. Our department engages in most of the clinical laboratory examinations in our hospital under stringent quality control and provides investigational laboratory analysis in collaboration with many other departments.
To facilitate translational research projects in this research hospital, we had established a special division named TR verification laboratory. This division has contributed to evaluating the safety of experimental therapeutic approaches and biopharmaceutical products for clinical trials.
As a central medical department, we are also taking part in many clinical trials and researches conducted in our hospital.

1. Introduction of various clinical laboratory tests for SARS-CoV-2 and environmental arrangements to prevent the infection in a clinical laboratory.

Microbiology team, Serology team, Physiology team, Tomohiro ISHIGAKI, and Tokiko NAGAMURA-INOUE

Due to the outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), prompt and accurate diagnosis of the infection (COVID-19) is essential for clinical practice.

Our clinical laboratory plays a vital role in the detection of the virus. The current standard method for diagnosing SARS-CoV-2 infection is the real-time reverse transcriptase-polymerase chain reaction (RT-PCR). To analyze more clinical samples and report the results faster, we have applied the BD MAX™ system, which is a fully-integrated automated platform that performs nucleic acid extraction and real-time PCR, to the clinical laboratory. We are reporting the results of various clinical samples within about 2 hours.

As SARS-CoV-2 continues to spread, emerging variants of the virus are being identified worldwide, and the identification of the variants gains importance. We, therefore, introduced RT-PCR melting temperature assay to rapidly screen for widely circulating SARS-CoV-2 variants and their mutation (such as N501Y, E484K, E484Q, L452R, S371L, and so on).

Moreover, vaccination for SARS-CoV-2 got gradually available this year. We have introduced SARS-CoV-2 antibody testing to analyze previous infection and immunity after vaccination.

Prevention of nosocomial infection is also important. This virus could quickly spread in confined spaces like a physiological laboratory. Respiratory

function tests and some physiological tests are at high risk for infection. Therefore, we advanced environmental arrangements to prevent the infection.

2. Prediction of deep vein thrombosis (DVT) based on laboratory results just before ultrasonography and improvement of the detection using a machine learning system.

Physiology team, Hematology team, Tomohiro ISHIGAKI, and Tokiko NAGAMURA-INOUE

Deep vein thrombosis (DVT) is venous thrombosis involving the formation of a blood clot in a deep vein, and sometimes causes a life-threatening condition, pulmonary embolism (PE). Early detection of DVT is essential, and ultrasonography plays a role in the detection. However, ultrasonography is not applicable to all patients as this test generally takes about 20-30 minutes. Hence, evaluation of pretest probability is recommended before the test according to the guideline of scientific societies.

We retrospectively analyzed the patients who received ultrasonography at our department and examined their coagulation fibrinolysis examination results before the ultrasonography. The result of each coagulation test is not enough to predict the existence of DVT (accuracy = 66.4% at maximum). We developed a deep neural network learning model, and deep learning of those laboratory results and general patient information could improve the predictive value (accuracy = 88.9%). The introduction of machine learning systems and optimization could make DVT detection more sensitive, and it would be useful to judge the necessity of ultrasonography. [Selected as one of chairman recommendation topics in the 68th annual meeting of JSLM / 2021]

3. Establishment and introduction of a new management system of clinical laboratory examinations for translational researches (TR).

TR verification laboratory team, Tomohiro ISHIGAKI, and Arinobu TOJO.

We have been supporting translational research (TR) by providing laboratory tests necessary to validate the safety of experimental therapeutic approaches and biopharmaceutical products for clinical trial studies like gene and cell therapies since 2008.

As these laboratory examinations, mainly for biopharmaceutical products, are different from usual clinical laboratory tests for patients, we had managed these laboratory tests by papers for more than a decade. This time, we tried to improve the management of laboratory orders, results, reports, and invoices by introducing a database server system. Clinical laboratory management systems commonly used for patient samples were not suitable for our purpose in cost and merit. We have completed the refinement at a low cost by introducing the commercially available database server system (Claris FileMaker, Santa Clara, CA, USA) and customizing the database to fit our clinical laboratory by ourselves. These efforts made our work more efficient, reduced human errors, enabled simultaneous sharing of results among various kinds of hospital workers.

The use of the database server system is valuable for the refinement of the management of laboratory results for translational research. [Article Accepted in 2021]

4. Laboratory contribution as a central medical department and support for many clinical investigations and trials in this hospital.

Hiroyuki SHINGYOCHI, Hironori SHIMOSAKA, and Clinical laboratory members (clinical hematology, biochemistry/serology, physiology, and microbiology team)

We participate in other clinical trials and research led by other hospital departments. Our laboratory members contributed to 8 clinical investigations and trials conducted in this hospital, including a test of new vaccination and a trial of treatment for COVID-19.

We also contributed to many basic and clinical studies.

IMSUT Hospital

Center for Clinical Safety and Infection Control
医療安全・感染制御センター

The Center for Clinical Safety and Infection Control consists of the Department of Medical Safety Management and the Department of Infection Prevention and Control and supports for providing safe medical care.

Department of Medical Safety Management
医療安全管理部

Head, Professor	Hiroshi Yotsuyanagi, M.D., D.M.Sc.	教　授	博士(医学)	四　柳　　宏
Associate Professor	Yoichi Imai, M.D., D.M.Sc.	准教授	博士(医学)	今　井　陽　一
Assistant Director	Junko Izumi	副看護部長		和　泉　純　子
Head Nurse	Hiromi Isshiki	看護師長		一　色　裕　美
Director of Pharmacy	Seiichiro Kuroda	薬剤部長		黒　田　誠一郎
Associate Professor	Ayako Kamisato, Ph.D.	准教授	博士(法学)	神　里　彩　子

Department Medical Safety Management consisting of doctors, a nurse, a pharmacist, and an administrative staff was founded in July 2001 and is responsible for carrying out medical safety in order to prevent incidents and accidents beforehand and deliver safe medical care to patients. Especially at our hospital, we mainly focus on hematological malignancies, infectious diseases, immune diseases, refractory malignant solid tumors etc, and are performing many kinds of therapies including transplantation. So we are keeping in mind that we can adequately respond to the things those will happen in these kinds of medical activities.

Department of Infection Prevention and Control
感染制御部

Head, Professor	Hiroshi Yotsuyanagi, M.D., D.M.Sc.	教　授	博士(医学)	四　柳　　宏
Assistant Professor	Eisuke Adachi, M.D., D.M.Sc.	助　教	博士(医学)	安　達　英　輔
Nurse Manager	Miya Kogayu	看護師長		小　粥　美　香
Pharmacist	Mika Yamamura	薬剤師		山　村　美　桂
Clinical laboratory technician	Hiroko Shibata	臨床検査技師		柴　田　浩　子

Department of Infection Prevention and Control builds ICT (Infection Control Team) and AST (Antimicrobial Stewardship Team) to promote the practice of hospital infection control and prevent the spread of antimicrobial resistant organisms. The ICT consists of infection control doctors, an infection control nurse, a pharmacist, a clinical laboratory technicians and an administrative staff.

An Outbreak of USA300 Methicillin-Resistant *Staphylococcus aureus* Among People With HIV in Japan

At a conference of Japan Infection Prevention and Control Conference for National in the Kanto area of Public University Hospitals, we reported on an outbreak of USA300 Methicillin-Resistant Staphylococcus aureus among people with HIV in Japan. We analyzed the cases of PVL-MRSA infection between 2010 and 2020 and screened for nasal colonization of PVL-MRSA in PWH who visited an HIV/AIDS referral hospital from December 2019 to March 2020. Whole-genome sequencing-based single nucleotide polymorphism analysis was performed on these isolates. The carriage prevalence was 4.3% (12/277) and PVL-MRSA carriers were more likely to have sexually transmitted infections (STIs) within a year compared with patients who had neither a history of PVL-MRSA infection. SNP analysis showed A history of STI was a risk of colonization.

Publication

1. Ikeuchi K, ○Adachi E, Sasaki T, Suzuki M, Yotsuyanagi H, et al. An outbreak of USA300 MRSA among people with HIV in Japan. J Infect Dis. 2021;223:610-20 .

IMSUT Hospital

Center for Translational Research
トランスレーショナルリサーチ・治験センター

Professor	Fumitaka Nagamura, M.D., D.M.Sc		教授	博士(医学)	長村文孝
Associate Professor	Masanori Nojima, M.D., Ph.D., M.P.H.		准教授	博士(医学)	野島正寛
Project Associate Professor	Hiroshi Yasui, M.D., Ph.D.		特任准教授	博士(医学)	安井寛

Our major mission is to support the conduct of clinical trials, especially for sponsor-investigator clinical trial based on Translational Research (TR). Our roles on TR varies from the advice for acquiring intellectual property, preparation for clinical trials, assistance for conducting clinical trials, and so on. Our center consists of coordinator section, administrative section, data management/biostatistics section, and project management section.

1. Promotion of Translational Research at IMSUT Hospital

All members of staff.

We have an unwavering commitment to deliver novel therapies through the conduct of translational research. To advance basic research findings into clinical application, we offer investigators the following services:

1)planning research and development (R & D) strategies, including selecting target diseases, planning product designs, and clarifying development pathways;
2)offering opportunities to consult an appointed patent attorney about acquisition and maintenance of intellectual property rights as well as patent strategies;
3)providing information necessary in preclinical phase of R & D, such as information on drug regulatory affairs and preclinical studies;
4)encouraging investigators to consult regulatory advisors of Pharmaceuticals and Medical Devices Agency (PMDA) in a timely manner;
5)participating in investigator-regulator meetings to help investigators deal with issues pointed out in the meetings;
6)advising on clinical trial design so that feasible and scientifically appropriate trials are conducted;
7)reviewing clinical study protocols, consent forms, and related documents in prior to Institutional Review Board examination to ensure the quality of clinical trials conducted at IMSUT Research Hospital;
8)assigning Translational Research Coordinators (TRCs) to each translational research project in the clinical trial phase; TRCs help patients participating in clinical trials to understand study protocols and to cope with negative emotions including fear, confusion, and depression; TRCs assist investigators.

2. Statistics and Quality control in Clinical Trials

Masanori Nojima, Motoki Amai, Mitsumi Tokunaga, Fumitaka Nagamura

We have planned and performed data management, monitoring, and statistical works in clinical trials.

[Data management]: Planning, EDC and CRF preparation, registration, allocation, database management, data cleaning, coding

[Monitoring]: Monitoring for drug management

[Statistics]: Planning and perform for statistical

analyses, Sample size calculation.

3. Support for the investigator-initiated clinical trials under an Investigational New Drug Application

All members of staff

Our mission is to develop efficient approaches for conducting investigator-initiated clinical trials under Investigational New Drug application (IND) to promote translational research. In 2021, we supported five sponsor-investigator clinical trials by site management as well as project management. These five clinical trials were: oncolytic virus for malignant melanoma, peptide therapy for after rejection of non-small cell lung cancer, phase II clinical trial with novel gene-induced adjuvant cells for acute myelogenous leukemia, nelfinavir for patients with COVID-19and booster administration of vaccine against COVID-19 infection.

4. Approach for epigenome and multi-omics research by methodology of bioinformatics and biostatistics

Masanori Nojima

Epigenome and multi-omics research using clinical samples in collaborative study or public database of comprehensive omics-analysis. We are now focusing on the multi-omics approach integrating DNA methylation, mRNA expression, and miRNA, and building statistical models to assess functional linkage.

5. Statistical consulting for basic research

Masanori Nojima

For basic researchers, we suggest exploratory statistical approach and molecular epidemiological approach.

6. Statistical consulting

Masanori Nojima

Consulting for study design and statistical analysis in any type of clinical research including clinical research, basic medical/biological research. We have collaborated with other members in IMSUT and other institutions through the consulting.

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IMSUT Hospital

Center for Antibody and Vaccine Therapy
抗体・ワクチンセンター

Professor	Hiroshi Yotsuyanagi, M.D., D.M.Sc.	教　授	博士(医学)	四　柳　　宏
Professor	Kouhei Tsumoto, Ph.D.	教　授	博士(工学)	津　本　浩　平
Project Associate Professor	Satoru Nagatoishi, Ph.D.	特任准教授	博士(生命科学)	長門石　　曉
Project Professor	Yataro Daigo, M.D., D.M.Sc.	特任教授	博士(医学)	醍　醐　弥太郎
Project Senior Assistant Professor	Atsushi Takano, M.D., D.M.Sc.	特任講師	博士(医学)	高　野　　淳
Project Assistant Professor	Michiko Koga, M.D., D.M.Sc.	特任助教	博士(医学)	古　賀　道　子

Our center was established in April 2012, in the memory of Professor Shibasaburo Kitasato, the founder and the first director of our institute, because the year 2012 was 120th anniversary of our institute which was built in 1892. Professor Kitasato was keen to utilize 'serum therapy' for patients with infectious diseases and actually developed therapeutic sera from horses. Now, we can use monoclonal antibodies to cytokines and their receptors, growth factor receptors, cellular kinases, for treatment of autoimmune diseases and cancer. The aim of this center is to develop novel immunological therapy for patients with various diseases including cancers, infectious diseases and autoimmune diseases. Moreover, attractive clinical trials are also ongoing in collaboration with research groups in IMSUT.

Daigo Group

1. Novel therapeutic target discovery for solid cancers

Yataro Daigo, Atsushi Takano, Koji Teramoto, Hidetoshi Sumimoto, Yoshinori Murakami, Phung Manh Thang, Kayo Daigo, Tomoyuki Igarashi, Masako Nakamura, Tsevegjav Bayarbat, Zhu Ming, Mbugua Regina Wachuka

To identify molecules involved in human carcinogenesis and those which could be applied for the development of new molecular therapies and/or biomarkers, we had established a systematic screening system as follows; i) identification of overexpressed and/or mutated genes in the majority of solid cancers (lung, esophagus, head-and-neck, breast, etc.) by genome-wide screening using the expression microarray as well as next generation sequencer in the combination of enrichment of tumor cell populations from cancer tissues by laser microdissection, ii) verification of no or little expression of each of candidate molecules in normal tissues by northern-blot analyses and expression microarray, iii) validation of the clinicopathological significance of its higher expression with tissue microarray containing thousands of archived solid cancers, iv) verification of a critical role of each target gene in the growth and/or invasiveness of cancer cells by RNAi and cell growth/invasion assays, v) evaluation of their usefulness as targets for passive immunotherapy using specific antibodies and/or as a serum biomarker for solid cancer by high throughput ELISA and proteomics analysis, if they are tumor-specific transmembrane or secretory proteins, vi) screening of the epitope peptides recognized by human histocompatibility leukocyte (HLA)-A*0201- or A*2402-restricted cytotoxic T lymphocyte (CTL) and dendritic

cell (DC). This systematic approach identified dozens of molecules that appear to fall into the category of oncoantigens or neoantigens whose overexpression is an important feature of the malignant nature of cancer cells and that have very high immunogenicity to induce antigen-specific CTLs in cancer patients. We further validated these molecules identified as potential targets for the development of antibodies, small-molecular compounds, growth-suppressive cell-permeable peptides, and cancer vaccines that could have a more specific and strong anti-cancer effect with minimal risk of adverse events. During this screening process, we found dozens of candidate molecules to be activated in various solid cancers including lung, esophagus, oral cavity, and breast cancers, as novel prognostic biomarkers and therapeutic targets.

2. Characterization of OIP5 as a biomarker and therapeutic target for oral cancer

Ming Zhu, Atsushi Takano, Bayarbat Tsevegjav, Yoshihiro Yoshitake, Masanori Shinohara, Yataro Daigo

In order to identify a novel prognostic biomarker and therapeutic target for oral cancer, we focused on the Opa interacting protein 5 (OIP5), which plays an essential role in the proper segregation of chromosome. Immunohistochemical analysis using tissue microarrays indicated that OIP5 was expressed in 120 of 164 (73.2%) oral cancers but was minimally expressed in normal oral tissues. OIP5 expression was significantly associated with poor prognosis for oral cancer. Overexpression of OIP5 enhanced the growth of oral cancer cells, whereas OIP5 knockdown using siRNAs significantly inhibited cell growth through cell cycle arrest at the G2/M phase. Suppression of OIP5 expression induced senescence of oral cancer cells. Our findings suggest that OIP5 may be a candidate prognostic biomarker and therapeutic target in oral cancer treatmet.

3. Development of therapeutic cancer vaccine

Yataro Daigo, Atsushi Takano, Koji Teramoto, Hidetoshi Sumimoto, Koichiro Yuji, Hiroshi Yasui, Giichiro Tsurita, Yoshihide Fujiyama, Kazumasa Ogasawara, Ikuo Toyama

Using the systematic screening system shown above, we identified concoantigens which were overexpressed in the majority of cancers derived from lung, esophagus and urinary bladder and essential for the growth and/or survival of cancer cells, as targets for therapeutic cancer vaccine treatment against various solid cancers. We screened dozens of 9- or 10-amino-acid epitope peptides recognized by human HLA-A*0201 and/or A*2402-restricted CTL by enzyme-linked immunospot (ELISPOT) assay. In IMSUT Hospital and its collaborative hospitals, International Conference on Harmonization (ICH) - Good Clinical Practice (GCP)-based clinical study using the combination of some of these peptides derived from oncoantigens in patients with lung cancer is now being conducted at 6 cancer centers and university hospitals including IMSUT hospital. In addition, new type of peptides-pulsed DC vaccination therapy is under development.

4. Integrated genomics-based discovery of new biomarkers for cancer immunotherapy and development T cell receptor-engineered T cell (TCR-T) therapy

Yataro Daigo, Atsushi Takano, Koji Teramoto, Hidetoshi Sumimoto, Koichiro Yuji, Hiroshi Yasui, Giichiro Tsurita, Yoshihide Fujiyama, Kazumasa Ogasawara, Ikuo Toyama, Yusuke Nakamura

Immune responses play a critical role in various disease conditions including cancer. Although various immunotherapies are being developed, predictive biomarkers for the choice of effective therapy are urgently required. Using systematic cancer genomics approach on clinical materials obtained from cancer patients treated with cancer vaccine, peptides-pulsed DC vaccination therapy, or immune checkpoint inhibitors, we are clarifying how molecular profiles of cancers can be used to identify biomarkers for predicting clinical outcomes. For example, there has not been a rapid, sensitive, comprehensive, and quantitative analysis method to examine T-cell or B-cell immune responses, therefore we developed a new approach to characterize tumor mutation burdens and T cell receptor (TCR) repertoire by sequencing millions of cDNA of exomes of cancer related genes as well as TCRα and β chains in combination with a newly-developed algorithm. Using samples from lung cancer patients, we are developing detailed information of neoantigen profiles of lung cancer patients and their TCR repertoire and are also developing T cell receptor-engineered T cell (TCR-T) therapy. We are now applying this next generation sequencing (NGS) platform to better understanding immune responses in many disease areas including immune disorders, allergies, and organ transplantations as well as development of new type of immunotherapies.

5. Detection of neoantigen-reactive T cell clones based on the clonal expansion using next-generation sequencing of TCRβ complementarity-determining region 3

Yataro Daigo, Hidetoshi Sumimoto, Koji Teramoto, Atsushi Takano

Development of mechanism-driven biomarkers

for immune checkpoint inhibitors in cancer immunotherapy requires sensitive and efficacious assays to identify tumor antigen (Ag)-specific T cells. We demonstrated the concept for a sensitive method to determine Ag-reactive T cell clones based on clonal expansion using model neoantigens rather than cytokine production. Sequential increase in T cell clonal frequencies following Ag stimulation was detected by NGS of TCRβ complementarity-determining region 3 (CDR3), with a higher sensitivity than that of ELISPOT assay by 100-fold. The TCRβ CDR3 sequences could represent useful markers to track dynamic changes during immunotherapy. We are validating TCRβ NGS-based method that could represent a novel platform both for the development of new biomarkers as well as several therapeutic options.

6. Scientific Platform of Supporting Cohort Study and Biospecimen Analysis

Yataro Daigo, Atsushi Takano, Koji Teramoto, Kohzoh Imai, Jun-ichiro Inoue, Yoshinori Murakami

To support life science researchers in the field of basic life science, cancer diagnostics and therapeutics, we are collecting cancer and corresponding normal tissues, serum, plasma, and peripheral blood mononuclear cell (PBMC) from patients with solid cancers originated from 30 organs. To date, we collected 113,700 clinical materials. We also constructed tissue microarray system covering about 5000 archived clinical cancers. Using these clinical materials, we are validating the clinicopathological significance of various candidate disease biomarkers as requested by researchers and are contributing to their clinical application and publications in international journals.

Publications

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Nagatoishi Group

Various types of antibodies have been approved for therapeutic use and currently examined in clinical development. Therefore, developments of technology for the discovery and optimization of high-potency antibodies have been improved and have greatly increased to find the specific and stable antibody with desired biological properties. Biophysical analyses of a therapeutic antibody, particularly those of protein interaction and stability, are recognized as one of the critical procedures in the development of biopharmaceuticals, which would be assessed as an essential step to develop next-generation antibodies. The development of analytical methods with quantitative and high-sensitive detection of antigen interaction, protein stability, and biological function of antibody, therefore, has been intriguing for the pharmaceutic companies. In this division, we study biophysical analyses of various antibodies to propose a new strategy for the development of the next-generation antibody.

1. Proteomic identification and validation of novel interactions of the putative tumor suppressor PRELP with membrane proteins including IGFI-R and p75NTR

Kosuge H, Nakakido M, Nagatoishi S, Fukuda T, Bando Y, Ohnuma SI, Tsumoto K.

Proline and arginine-rich end leucine-rich repeat protein (PRELP) is a member of the small leucine-rich repeat proteoglycans (SLRPs) family. Levels of PRELP mRNA are downregulated in many types of cancer, and PRELP has been reported to have suppressive effects on tumor cell growth, although the molecular mechanism has yet to be fully elucidated. Given that other SLRPs regulate signaling pathways through interactions with various membrane proteins, we reasoned that PRELP likely interacts with membrane proteins to maintain cellular homeostasis. To identify membrane proteins that interact with PRELP, we carried out coimmunoprecipitation coupled with mass spectrometry (CoIP-MS). We prepared membrane fractions from Expi293 cells transfected to overexpress FLAG-tagged PRELP or control cells and analyzed samples precipitated with anti-FLAG antibody by mass spectrometry. Comparison of membrane proteins in each sample identified several that seem to interact with PRELP; among them, we noted two growth factor receptors, insulin-like growth factor I receptor (IGFI-R) and low-affinity nerve growth factor receptor (p75NTR), interactions with which might help to explain PRELP's links to cancer. We demonstrated that PRELP directly binds to extracellular domains of these two growth factor receptors with low micromolar affinities by surface plasmon resonance analysis using recombinant proteins. Furthermore,

cell-based analysis using recombinant PRELP protein showed that PRELP suppressed cell growth and affected cell morphology of A549 lung carcinoma cells, also at micromolar concentration. These results suggest that PRELP regulates cellular functions through interactions with IGFI-R and p75NTR and provide a broader set of candidate partners for further exploration

2. Heme controls the structural rearrangement of its sensor protein mediating the hemolytic bacterial survival

Nishinaga M, Sugimoto H, Nishitani Y, Nagai S, Nagatoishi S, Muraki N, Tosha T, Tsumoto K, Aono S, Shiro Y, Sawai H.

Hemes (iron-porphyrins) are critical for biological processes in all organisms. Hemolytic bacteria survive by acquiring b-type heme from hemoglobin in red blood cells from their animal hosts. These bacteria avoid the cytotoxicity of excess heme during hemolysis by expressing heme-responsive sensor proteins that act as transcriptional factors to regulate the heme efflux system in response to the cellular heme concentration. Here, the underlying regulatory mechanisms were investigated using crystallographic, spectroscopic, and biochemical studies to understand the structural basis of the heme-responsive sensor protein PefR from Streptococcus agalactiae, a causative agent of neonatal life-threatening infections. Structural comparison of heme-free PefR, its complex with a target DNA, and heme-bound PefR revealed that unique heme coordination controls a >20 Å structural rearrangement of the DNA binding domains to dissociate PefR from the target DNA. We also found heme-bound PefR stably binds exogenous ligands, including carbon monoxide, a by-product of the heme degradation reaction.

3. A DNA Aptamer That Inhibits the Aberrant Signaling of Fibroblast Growth Factor Receptor in Cancer Cells

Eguchi A, Ueki A, Hoshiyama J, Kuwata K, Chikaoka Y, Kawamura T, Nagatoishi S, Tsumoto K, Ueki R, Sando S.

Growth factor receptors are activated through dimerization by the binding of their ligands and play pivotal roles in normal cell function. However, the aberrant activity of the receptors has been associated with cancer malignancy. One of the main causes of the aberrant receptor activation is the overexpression of receptors and the resultant formation of unliganded receptor dimers, which can be activated in the absence of external ligand molecules. Thus, the unliganded receptor dimer is a promising target to inhibit aberrant signaling in cancer. Here, we report an aptamer that specifically binds to fibroblast growth factor receptor 2b and inhibits the aberrant receptor activation and signaling. Our investigation suggests that this aptamer inhibits the formation of the receptor dimer occurring in the absence of external ligand molecules. This work presents a new inhibitory function of aptamers and the possibility of oligonucleotide-based therapeutics for cancer.

4. Thermodynamic Dissection of Potency and Selectivity of Cytosolic Hsp90 Inhibitors.

Yoshimura C, Nagatoishi S, Kuroda D, Kodama Y, Uno T, Kitade M, Chong-Takata K, Oshiumi H, Muraoka H, Yamashita S, Kawai Y, Ohkubo S, Tsumoto K.

The cytosolic Hsp90-selective inhibitor TAS-116 has an acceptable safety profile and promising antitumor activity in clinical trials. We examined the binding characteristics of TAS-116 and its analogs to determine the impact of the ligand binding mode on selectivity for cytosolic Hsp90. Analyses of the co-crystal structure of Hsp90 and inhibitor TAS-116 suggest that TAS-116 interacts with the ATP-binding pocket, the ATP lid region, and the hydrophobic pocket. A competitive isothermal titration calorimetry analysis confirmed that a small fragment of TAS-116 (THS-510) docks into the lid region and hydrophobic pockets without binding to the ATP-binding pocket. THS-510 exhibited enthalpy-driven binding to Hsp90α and selectively inhibited cytosolic Hsp90 activity. The heat capacity change of THS-510 binding was positive, likely due to the induced conformational rearrangement of Hsp90. Thus, we concluded that interactions with the hydrophobic pocket of Hsp90 determine potency and selectivity of TAS-116 and derivatives for the cytosolic Hsp90 isoform

5. Elaboration of Non-naturally Occurring Helical Tripeptides as p53-MDM2/MDMX Interaction Inhibitors

Su A, Tabata Y, Aoki K, Sada A, Ohki R, Nagatoishi S, Tsumoto K, Wang S, Otani Y, Ohwada T.

Protein-protein interactions (PPIs) are often mediated by helical, strand and/or coil secondary structures at the interface regions. We previously showed that non-naturally occurring, stable helical trimers of bicyclic β-amino acids (Abh) with all-trans amide bonds can block the p53-MDM2/MDMX α-helix-helix interaction, which plays a role in regulating p53 function. Here, we conducted docking and molecular dynamics calculations to guide the structural optimization of our reported compounds, focusing on modifications of the C-terminal/N-terminal residues. We confirmed that the modified peptides directly bind to MDM2 by means of thermal shift assay, iso-

thermal titration calorimetry, and enzyme-linked immunosorbent assay (ELISA) experiments. Biological activity assay in human osteosarcoma cell line SJSA-1, which has wild-type p53 and amplification of the Mdm2 gene, indicated that these peptides are membrane-permeable p53-MDM2/MDMX interaction antagonists that can rescue p53 function in the cells.

6. Anion solvation enhanced by positive supercharging mutations preserves thermal stability of an antibody in a wide pH range

Kasahara K, Kuroda D, Tanabe A, Kawade R, Nagatoishi S, Tsumoto K.

Proteins function through interactions with other molecules. In protein engineering, scientists often engineer proteins by mutating their amino acid sequences on the protein surface to improve various physicochemical properties. "Supercharging" is a method to design proteins by mutating surface residues with charged amino acids. Previous studies demonstrated that supercharging mutations conferred better thermal resistance, solubility, and cell penetration to proteins. Likewise, antibodies recognize antigens through the antigen-binding site on the surface. The genetic and structural diversity of antibodies leads to high specificity and affinity toward antigens, enabling antibodies to be versatile tools in various applications. When assessing therapeutic antibodies, surface charge is an important factor to consider because the isoelectric point plays a role in protein clearance inside the body. In this study, we explored how supercharging mutations affect physicochemical properties of antibodies. Starting from a crystal structure of an antibody with the net charge of -4, we computationally designed a supercharged variant possessing the net charge of +10. The positive-supercharged antibody exhibited marginal improvement in thermal stability, but the secondary structure and the binding affinity to the antigen (net charge of +8) were preserved. We also used physicochemical measurements and molecular dynamics simulations to analyze the effects of supercharging mutations in sodium phosphate buffer with different pH and ion concentrations, which revealed preferential solvation of phosphate ions to the supercharged surface relative to the wild-type surface. These results suggest that supercharging would be a useful approach to preserving thermal stability of antibodies in a wide range of pH, which may enable further diversification of antibody repertoires beyond natural evolution.

7. A glutamine sensor that directly activates TORC1

Tanigawa M, Yamamoto K, Nagatoishi S, Nagata K, Noshiro D, Noda NN, Tsumoto K, Maeda T.

TOR complex 1 (TORC1) is an evolutionarily-conserved protein kinase that controls cell growth and metabolism in response to nutrients, particularly amino acids. In mammals, several amino acid sensors have been identified that converge on the multi-layered machinery regulating Rag GTPases to trigger TORC1 activation; however, these sensors are not conserved in many other organisms including yeast. Previously, we reported that glutamine activates yeast TORC1 via a Gtr (Rag ortholog)-independent mechanism involving the vacuolar protein Pib2, although the identity of the supposed glutamine sensor and the exact TORC1 activation mechanism remain unclear. In this study, we successfully reconstituted glutamine-responsive TORC1 activation in vitro using only purified Pib2 and TORC1. In addition, we found that glutamine specifically induced a change in the folding state of Pib2. These findings indicate that Pib2 is a glutamine sensor that directly activates TORC1, providing a new model for the metabolic control of cells.

8. Mechanism of dimerization and structural features of human LI-cadherin

Yui A, Caaveiro JMM, Kuroda D, Nakakido M, Nagatoishi S, Goda S, Maruno T, Uchiyama S, Tsumoto K

Liver intestine (LI)-cadherin is a member of the cadherin superfamily, which encompasses a group of Ca2 + -dependent cell-adhesion proteins. The expression of LI-cadherin is observed on various types of cells in the human body, such as normal small intestine and colon cells, and gastric cancer cells. Because its expression is not observed on normal gastric cells, LI-cadherin is a promising target for gastric cancer imaging. However, because the cell adhesion mechanism of LI-cadherin has remained unknown, rational design of therapeutic molecules targeting this cadherin has been hampered. Here, we have studied the homodimerization mechanism of LI-cadherin. We report the crystal structure of the LI-cadherin homodimer containing its first four extracellular cadherin repeats (EC1-4). The EC1-4 homodimer exhibited a unique architecture different from that of other cadherins reported so far, driven by the interactions between EC2 of one protein chain and EC4 of the second protein chain. The crystal structure also revealed that LI-cadherin possesses a noncanonical calcium ion-free linker between the EC2 and EC3 domains. Various biochemical techniques and molecular dynamics simulations were employed to elucidate the mechanism of homodimerization. We also showed that the formation of the homodimer observed in the crystal structure is necessary for LI-cadherin-dependent cell adhesion by performing cell aggregation assays. Taken together, our data provide structural insights necessary to advance the use of LI-cadherin as

a target for imaging gastric cancer.

9. Regulation of cadherin dimerization by chemical fragments as a trigger to inhibit cell adhesion

Senoo A, Ito S, Nagatoishi S, Saito Y, Ueno G, Kuroda D, Yoshida K, Tashima T, Kudo S, Sando S, Tsumoto K.

Many cadherin family proteins are associated with diseases such as cancer. Since cell adhesion requires homodimerization of cadherin molecules, a small-molecule regulator of dimerization would have therapeutic potential. Herein, we describe identification of a P-cadherin-specific chemical fragment that inhibits P-cadherin-mediated cell adhesion. Although the identified molecule is a fragment compound, it binds to a cavity of P-cadherin that has not previously been targeted, indirectly prevents formation of hydrogen bonds necessary for formation of an intermediate called the X dimer and thus modulates the process of X dimerization. Our findings will impact on a strategy for regulation of protein-protein interactions and stepwise assembly of protein complexes using small molecules.

10. Development of biparatopic bispecific antibody possessing tetravalent scFv-Fc capable of binding to ROBO1 expressed in hepatocellular carcinoma cells

Watanabe Y, Tanabe A, Hamakubo T, Nagatoishi S, Tsumoto K.

There is no standard structural format of the biparatopic bispecific antibody (bbsAb) which is used against the target molecule because of the diversity of biophysical features of bispecific antibodies (bsAbs). It is therefore essential that the interaction between the antibody and antigen is quantitatively analyzed to design antibodies that possess the desired properties. Here, we generated bsAbs, namely, a tandem scFv-Fc, a diabody-Fc, and an immunofusion-scFv-Fc-scFv, that possessed four scFv arms at different positions and were capable of recognizing the extracellular domains of ROBO1. We examined the interactions between these bsAbs and ROBO1 at the biophysical and cellular levels. Of these, immunofusion-B2212A scFv-Fc-B5209B scFv was stably expressed with the highest relative yield. The kinetic and thermodynamic features of the interactions of each bsAb with soluble ROBO1 (sROBO1) were validated using surface plasmon resonance and isothermal titration calorimetry. In all bsAbs, the immunofusion-scFv-Fc-scFv format showed homogeneous interaction with the antigen with higher affinity compared with that of monospecific antibodies. In conclusion, our study presents constructive information to design druggable bbsAbs in drug applications.

11. Electrostatic-triggered exothermic antibody adsorption to the cellulose nanoparticles

Murakami K, Nagatoishi S, Kasahara K, Nagai H, Sasajima Y, Sasaki R, Tsumoto K.

Antibody-conjugated nanoparticles are used in a fields ranging from medicine to engineering. NanoAct® nanobeads are cellulose nanoparticles used in lateral flow assays that are highly water dispersible. In order to promote the adsorption of antibodies onto NanoAct® particles while maintaining their activity, we analyzed the adsorption onto NanoAct® particles thermodynamically and elucidated the adsorption mechanism. In an immunochromatographic assay, the amount of adsorbed antibody and the color intensity of the test line increased as the pH decreased. The zeta potential of the nanoparticles remained constant at around -30 mV over the pH range from 2 to 10. The model antibody had pI values between 6.2 and 6.8. Isothermal calorimetry analysis showed that adsorption of antibody to the NanoAct® particle is an endothermic reaction under low pH conditions, an exothermic reaction between pH 6 and pH 7, and a weakly exothermic reaction above pH 7. These data indicate that the changes in net charge of the antibody surface as a function of pH influence the pH dependence of antibody adsorption to the negatively charged NanoAct®. This suggests that increased positive charge on the antibody surface will result in a more sensitive NanoAct®-based immunoassay.

12. An integrated computational pipeline for designing high-affinity nanobodies with expanded genetic codes

Padhi AK, Kumar A, Haruna KI, Sato H, Tamura H, Nagatoishi S, Tsumoto K, Yamaguchi A, Iraha F, Takahashi M, Sakamoto K, Zhang KYJ.

Protein engineering and design principles employing the 20 standard amino acids have been extensively used to achieve stable protein scaffolds and deliver their specific activities. Although this confers some advantages, it often restricts the sequence, chemical space, and ultimately the functional diversity of proteins. Moreover, although site-specific incorporation of non-natural amino acids (nnAAs) has been proven to be a valuable strategy in protein engineering and therapeutics development, its utility in the affinity-maturation of nanobodies is not fully explored. Besides, current experimental methods do not routinely employ nnAAs due to their enormous library size and infinite combinations. To address this, we have developed an integrated computational pipeline employing structure-based protein design methodologies, molecular dynamics simulations and

free energy calculations, for the binding affinity prediction of an nnAA-incorporated nanobody toward its target and selection of potent binders. We show that by incorporating halogenated tyrosines, the affinity of 9G8 nanobody can be improved toward epidermal growth factor receptor (EGFR), a crucial cancer target. Surface plasmon resonance (SPR) assays showed that the binding of several 3-chloro-l-tyrosine (3MY)-incorporated nanobodies were improved up to 6-fold into a picomolar range, and the computationally estimated binding affinities shared a Pearson's r of 0.87 with SPR results. The improved affinity was found to be due to enhanced van der Waals interactions of key 3MY-proximate nanobody residues with EGFR, and an overall increase in the nanobody's structural stability. In conclusion, we show that our method can facilitate screening large libraries and predict potent site-specific nnAA-incorporated nanobody binders against crucial disease-targets.

13. The transcriptional corepressor CtBP2 serves as a metabolite sensor orchestrating hepatic glucose and lipid homeostasis

Ota T, Senoo A, Shirakawa M, Nonaka H, Saito Y, Ito S, Ueno G, Nagatoishi S, Tsumoto K, Sando S.

Biological systems to sense and respond to metabolic perturbations are critical for the maintenance of cellular homeostasis. Here we describe a hepatic system in this context orchestrated by the transcriptional corepressor C-terminal binding protein 2 (CtBP2) that harbors metabolite-sensing capabilities. The repressor activity of CtBP2 is reciprocally regulated by NADH and acyl-CoAs. CtBP2 represses Forkhead box O1 (FoxO1)-mediated hepatic gluconeogenesis directly as well as Sterol Regulatory Element-Binding Protein 1 (SREBP1)-mediated lipogenesis indirectly. The activity of CtBP2 is markedly defective in obese liver reflecting the metabolic perturbations. Thus, liver-specific CtBP2 deletion promotes hepatic gluconeogenesis and accelerates the progression of steatohepatitis. Conversely, activation of CtBP2 ameliorates diabetes and hepatic steatosis in obesity. The structure-function relationships revealed in this study identify a critical structural domain called Rossmann fold, a metabolite-sensing pocket, that is susceptible to metabolic liabilities and potentially targetable for developing therapeutic approaches

14. Structural and thermodynamical insights into the binding and inhibition of FIH-1 by the N-terminal disordered region of Mint3

Ten T, Nagatoishi S, Maeda R, Hoshino M, Nakayama Y, Seiki M, Sakamoto T, Tsumoto K.

Mint3 is known to enhance aerobic ATP production, known as the Warburg effect, by binding to FIH-1. Since this effect is considered to be beneficial for cancer cells, the interaction is a promising target for cancer therapy. However, previous research has suggested that the interacting region of Mint3 with FIH-1 is intrinsically disordered, which makes investigation of this interaction challenging. Therefore, we adopted thermodynamic and structural studies in solution to clarify the structural and thermodynamical changes of Mint3 binding to FIH-1. First, using a combination of circular dichroism, nuclear magnetic resonance, and hydrogen/deuterium exchange-mass spectrometry (HDX-MS), we confirmed that the N-terminal half, which is the interacting part of Mint3, is mostly disordered. Next, we revealed a large enthalpy and entropy change in the interaction of Mint3 using isothermal titration calorimetry (ITC). The profile is consistent with the model that the flexibility of disordered Mint3 is drastically reduced upon binding to FIH-1. Moreover, we performed a series of ITC experiments with several types of truncated Mint3s, an effective approach since the interacting part of Mint3 is disordered, and identified amino acids 78 to 88 as a novel core site for binding to FIH-1. The truncation study of Mint3 also revealed the thermodynamic contribution of each part of Mint3 to the interaction with FIH-1, where the core sites contribute to the affinity (ΔG), while other sites only affect enthalpy (ΔH), by forming noncovalent bonds. This insight can serve as a foothold for further investigation of intrinsically disordered regions (IDRs) and drug development for cancer therapy.

15. Structure-based screening combined with computational and biochemical analyses identified the inhibitor targeting the binding of DNA Ligase 1 to UHRF1

Kori S, Shibahashi Y, Ekimoto T, Nishiyama A, Yoshimi S, Yamaguchi K, Nagatoishi S, Ohta M, Tsumoto K, Nakanishi M, Defossez PA, Ikeguchi M, Arita K.

The accumulation of epigenetic alterations is one of the major causes of tumorigenesis. Aberrant DNA methylation patterns cause genome instability and silencing of tumor suppressor genes in various types of tumors. Therefore, drugs that target DNA methylation-regulating factors have great potential for cancer therapy. Ubiquitin-like containing PHD and RING finger domain 1 (UHRF1) is an essential factor for DNA methylation maintenance. UHRF1 is overexpressed in various cancer cells and down-regulation of UHRF1 in these cells reactivates the expression of tumor suppressor genes, thus UHRF1 is a promising target for cancer therapy. We have previously shown that interaction between the tandem Tudor domain (TTD) of UHRF1 and DNA ligase 1 (LIG1) di/trimethylated on Lys126 plays a key role in the recruitment of UHRF1 to replication sites and replication-coupled

DNA methylation maintenance. An arginine binding cavity (Arg-binding cavity) of the TTD is essential for LIG1 interaction, thus the development of inhibitors that target the Arg-binding cavity could potentially repress UHRF1 function in cancer cells. To develop such an inhibitor, we performed in silico screening using not only static but also dynamic metrics based on all-atom molecular dynamics simulations, resulting in efficient identification of 5-amino-2,4-dimethylpyridine (5A-DMP) as a novel TTD-binding compound. Crystal structure of the TTD in complex with 5A-DMP revealed that the compound stably bound to the Arg-binding cavity of the TTD. Furthermore, 5A-DMP inhibits the full-length UHRF1:LIG1 interaction in Xenopus egg extracts. Our study uncovers a UHRF1 inhibitor which can be the basis of future experiments for cancer therapy.

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IMSUT Hospital

Therapeutic Vector Development Center
治療ベクター開発センター

Professor	Tomoki Todo, M.D., Ph.D.	教　授	博士（医学）	藤　堂　具　紀
Project Associate Professor	Minoru Tanaka, M.D., Ph.D.	特任准教授	博士（医学）	田　中　　実

The Therapeutic Vector Development Center (TVDC), formerly named Core Facility for Therapeutic Vectors, was established in 2002 as the first facility in Japanese academia for the clinical-grade production of viral or cellular vectors. TVDC is designed to support clinical trials that require the production of recombinant viral vectors, genetic modification, and/or ex vivo manipulation of patient-derived tissues or cells under current Good Manufacturing Practice (cGMP) conditions.

Maintenance of the Standard Operating Procedures (SOPs)

The cGMP compliance is maintained by the regularly-revised SOPs that document all the elements of laboratory works, including both tangible and intangible factors like equipment, facility design, personnel, etc.

ISO certification

The management system of TVDC was re-qualified as ISO9001-certified in 2020, which has been regularly performed by an independent organization to meet the requirement for ISO9001 standard.

Validation of TVDC

The TVDC consists of two units; 1) Vector Unit, the primary suite for viral vector production and ex vivo transduction; 2) Cell Unit, the suite for cell processing capable of generating therapeutic cells such as dendritic cells for immunotherapy and gene therapy. Each unit has two independent compartments kept as a Class 10,000 clean level. The facility and equipment are regularly validated by the SOPs to fulfill the cGMP standard.

Production of clinical grade oncolytic HSV-1

Multiple lots of clinical-grade oncolytic herpes simplex virus type 1 (HSV-1) have been produced in the Vector Unit by the laboratory specialists of the Division of Innovative Cancer Therapy.

Oncolytic MV project

A clinical-grade oncolytic measles virus (MV) was produced by the Laboratory Animal Research Center and stored in the Vector Unit.

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IMSUT Hospital

IMSUT CORD
臍帯血・臍帯バンク

Clinical Professor　Tokiko Nagamura-Inoue, M.D., Ph.D.　病院教授　博士（医学）　長　村　登紀子

Recently, umbilical cord blood (CB) has received attention as the optimum allogeneic source for immunotherapies. More recently, the umbilical cord (UC) has been rapidly utilized as an abundant source of mesenchymal stromal cells (MSCs), which migrate toward inflamed or damaged tissue to reduce inflammation and support tissue repair. Both CB and UC can be provided as "off-the-shelf" cell products for immunotherapies and regenerative medicine. IMSUT CORD is the CB- and UC-derived cell bank established in the Institute of Medical Science, University of Tokyo (IMSUT) hospital in 2016. The aim of IMSUT CORD is to collect, process/culture, cryopreserve, stock, and release CB- and UC-derived cells—including mesenchymal stromal cells (MSCs)—for clinical and research use. We have released CB and UC-derived MSCs to researchers under material transfer agreements to expedite translational studies in the immunotherapy, regenerative medicine, and disease-specific drug-discovery fields. We have supplied UC-MSC products for clinical trials for severe acute graft-versus-host disease (GVHD; 2018–2020), COVID-19-related ARDS (2020–), and cerebral palsy (PVL; 2021–). We are currently preparing the UC-MSC product for a clinical trial for treating non-infectious pulmonary complications after hematopoietic stem cell transplantation.

1. Establishing a stable perinatal appendage–derived cell supply system as the source of allogeneic somatic stem cells for research and clinical use

Nagamura-Inoue T, Takahashi A, Hori A, Miharu Y, Yamamoto Y, Iwasawa N, Nagaya N, Ogami K, Okamura K, Mukai T, Nagamura F

The umbilical cord (UC) is a rich source of mesenchymal stromal cells (MSCs). UC-derived MSCs (UC-MSCs) possess many advantages: (1) ease of collection, storage, and transport; (2) abundant sources with high proliferation capacity; (3) multipotency to differentiate into various tissue cells, including osteoblasts, chondroblasts, adipocytes, and neurons; (4) low immunogenicity with significant immunosuppressive ability; (5) tissue repair potency; (6) ability to migrate toward the site of inflammation or injury, thereby reducing inflammation and repairing damaged tissues, and (7) no donor age–dependent variations.

We established a cord/cord-blood bank at the IMSUT hospital (IMSUT CORD) to collect cord blood (CB) and UC tissue (after informed consent from the mothers in collaboration with the obstetricians), to freeze UC, and to manufacture master cells and product cells for research and clinical use. For clinical use, we introduced a serum-free process throughout the manufacture.

To maintain quality control, we introduced the ISO 9001:2015 quality management standards in IMSUT CORD from 2018. We have transferred the manufacturing and testing technologies to the client companies, where they apply our techniques and standards in their clinical trials—including therapies

for acute GVHD, cerebral palsy, and COVID-19–related acute respiratory distress syndrome (ARDS).

The IMSUT CORD mission is to establish a supply system for UC-MSCs as a source of allogeneic somatic stem cells in research and clinical use. We supply UC-MSCs for research use in developing cell therapies. In addition, we have supplied clinical-grade UC-MSC products (namely IMSUT-CORD) for clinical treatment trials for severe acute graft-versus-host disease (GVHD; 2018–2020), COVID-19–related ARDS (2020–), and cerebral palsy (PVL; 2021–) after approval by the review board. We are currently preparing the UC-MSC product for a clinical trial for treating non-infectious pulmonary complications after hematopoietic stem cell transplantation.

In 2021, our main manufacturing location moved from the IMSUT-Cell Resource Center (IMSUT-CRC) to a new facility, the IMSUT-HLC Cell Processing Facility (IMSUT-HLC CPF), while other functions—including quality testing, documentation, and information control—remain in IMSUT-CRC

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看護師長		都留由香里
看護師長		亀田史絵

Department of Nursing seeks to provide high-quality nursing care and contribute to the team approach to patient centered care to meet diversified needs, along with changes in social circumstances and with the progress of medical science. In 2021, we focused on responding to the new coronavirus infection (COVID-19), which shows no signs of convergence, and providing nursing in response to the expansion of medical care.

Under the mission of "Making a difference in patient outcomes with the power of nursing," we nurses aim to provide optimal nursing so that patients can receive high-quality treatment. We would like to support patients so that they can respect their social lives and lead meaningful lives. To that end, as part of our medical team, we want to do our best to protect the dignity and safety of our patients and improve medical care and quality of life.

In 2021 began with the third wave of COVID-19. In late March, medical personnel began vaccination COVID-19, and the Nursing Department cooperated as an injection officer.

The second vaccination ended in May. The fourth wave came during the vaccination period, but it converged in late May, and the zoning of infectious disease wards was eased from early June .Medical personnel can enter the staff station of the infectious disease ward with a surgical mask. The fifth wave arrived in late July, and the number of infected people reached a record high in August, tight medical care. Our hospital also accepted the patient of the mild to the moderate every day. It overlapped with the summer vacation period, and it was difficult to respond only to nurses in infectious disease wards, so we secured human resources by dispatching support from other departments. There was no shortage of sanitary materials, but there was a shortage of medical equipment related to oxygen therapy, and we responded by rental. In order to accept moderate COVID-19 patients, we held a study session on respiratory care in the ward so that each nurse could provide nursing according to the patient's respiratory condition. He was also actively involved in patient guidance with an eye on life after discharge. The fifth wave converged in late September, and we were able to safely overcome it through multi-disciplinary collaboration including doctors. From October to early November,

there were no inpatients with COVID-19, and from late November, general patients were hospitalized in a section of infectious disease wards.

In the department of surgical systems, the number of surgeries increased due to the expansion of indications for robot-assisted surgery (robot-assisted anterior rectal resection, robot-assisted kidney partial resection), total cystectomy in urology, and the start of viral therapy for brain tumor surgery. In the two surgical wards, information on surgery was shared at study sessions and surgical conferences, and efforts were made to standardize nursing practices so that patients in both wards could receive similar nursing regardless of whether they were hospitalized. In addition, nurses belonging to outpatient clinics, wards, and operating rooms cooperated to review perioperative nursing standards and care pathways so that patients can undergo surgery with peace of mind. As a result, standardization of perioperative nursing advances, and the role in each nursing unit becomes clear, and it is expected to lead to the provision of stable nursing.

However, with the expansion of medical care, treatment with risks has also increased, and the number of patients requiring intensive care has increased from last year. Since there are multiple nurses in each nursing unit who worked in intensive care units at other hospitals, there is no problem in emergency response. In the absence of them, it is predicted that emergency response will weaken. Therefore, nurses with nursing experience in intensive care units voluntarily hold study sessions on nursing and emergency response during ventilator management, and provide patient care together to contribute to the improvement of intensive care. Nurses who have never had experience in intensive care can provide care with peace of mind by acquiring knowledge and skills and experiencing patient care together, leading to confidence as a nurse.

Our hospital is a medical science laboratory hospital that is responsible for the development of advanced medical care, and responds to clinical trials, the latest treatment and examination, and infection epidemics. Based on this current situation, we will be aware of the risks associated with medical care, and we would like to establish a nursing system so that we can provide warm medical care and nursing to patients in the future.

Publication

一色裕美，小澤昌子，小粥美香，都留由香里，リンツビヒラ希，久原みな代，吉井栄子．副看護師長と看護師が発揮するコンピテンシーの関連性の検討．看護管理．31(3)：217-221，2021

小粥美香．病棟から外来へ！看護計画の引き継ぎと展開．外来看護．26(3)秋号：12-17，2021

Conference Presentation

織田ひとみ，佐藤朋子，高坂久仁子，松下はるゑ，宮本幸奈，谷井真弓．臨床研究看護の質向上のための取り組み―臨床研究における専門職連携推進を目指して―．第35回日本がん看護学会学術集会．2021. 2. 27～4. 30

高坂久仁子，佐藤朋子，松下はるゑ，宮本幸奈，織田ひとみ，谷井真弓．臨床研究看護の質向上のための取り組み―看護基準活用のためにディスカッション形式の学習会を企画して―．第35回日本がん看護学会学術集会．2021. 2. 27～4. 30

松下はるゑ，宮本幸奈，佐藤朋子，織田ひとみ，高坂久仁子，谷井真弓．臨床研究看護の質向上のための取り組み―患者と家族のニーズを踏まえた意思決定支援拡充に向けて―．第35回日本がん看護学会学術集会．2021. 2. 27～4. 30

都留由香里，砂田純子，吉井栄子．輸液を安全に実施するための時間確保について～日勤始業後1時間の業務改善～．第7回日本医療安全学会学術総会2021. 5. 29～6. 7（Web開催）

成田初子．自己管理薬の内服間違い低減への取り組み．第7回日本医療安全学会学術集会2021. 5. 29～6. 7（Web開催）

山口恭子，小粥美香，小林路世．RARP時の皮膚トラブルや神経障害予防への取り組み．第32回日本手術看護学会 関東甲信越地区学会2021. 6. 26～7. 25（Web開催）（オンデマンド）

小粥美香，杉山栄美子，小澤昌子，和泉純子，吉井栄子．看護記録の内容監査とラダー研修・現場教育の連携を目指した取り組み．第25回日本看護管理学会学術集会．横浜．2021. 8. 28～29（ハイブリッド開催）（オンデマンド配信8. 28～9. 27）

リンツビヒラ希，一色裕美，小粥美香，成田初子，和泉純子，久原みな代，吉井栄子．コンピテンシーと課題解決技手法を活用した新規診療科開設の取り組み．第25回日本看護管理学会学術集会．横浜．2021. 8. 28～29.（ハイブリッド開催）（オンデマンド配信8. 28～9. 27）

鮫江真帆，中水流彩，中村伸枝．学童後期から青年期までの患児の院内学級の体験を支えるための看護援助．千葉看護学会第27回学術集会．2021. 9. 4（Web開催）

小野谷厚子．COVID-19専用病棟の開設時の取り組み．第36回日本環境感染学会総会・学術集会．名古屋．2021. 9. 19～20

亀田史絵．新型コロナウイルス感染症に対する当院の感染対策の活動報告．　第36回日本環境感染学会総会・学術集会．名古屋．2021. 9. 19～20

小粥美香，亀田史絵，山村実佳，古賀道子，安達英

輔，堤武也，四柳宏．新型コロナワクチン接種後の副反応について報告．第36回日本環境感染学会総会・学術集会．名古屋．2021. 9. 19～20

IMSUT Hospital

Department of Pharmacy
薬剤部

Director	Seiichiro Kuroda
Pharmacist	Sonoe Higasino
Pharmacist	Yohei Iimura
Pharmacist	Mika Yamamura
Pharmacist	Mai Yokota

薬剤部長	黒田誠一郎
薬剤師	東野園恵
薬剤師	飯村洋平
薬剤師	山村実佳
薬剤師	横田舞

The Department of Pharmacy seeks to provide high-quality pharmaceutical care services. We contribute to the team approach to patient-oriented medical care and provides a drug distribution services. We are also trying to contribute to propel the right use of medicines for patients.

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2) **Iimura, Y,. Kurokawa, T,. Andoh, S,. Kanemoto, Y,. Kawamata, T,. Yazawa, K,. Sato, A,. Yokoyama, K,. Imai, Y,. Tsurita, G,. Ahiko, Y,. Aikou, S,. Shida, D,. Nojima, M,. Tojo, A,. Sugiura, M. and Kuroda, S. Association between thiamine decrease and neuropsychiatric symptoms in gastrointestinal and hematological cancer patients receiving chemotherapy. Biomed Pharmacother. 141:111929. doi: 10.1016/j.biopha.2021.111929, 2021.**

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4) **Iimura, Y,. Kurokawa, T,. Kanemoto, Y,. Yazawa, K,. Tsurita, G,. Ahiko, Y,. Aikou, S,. Shida, D. and Kuroda, S. Severe thrombocytopenia induced by chemotherapy after total gastrectomy: A report of three cases. Int J Clin Pharmacol Ther. Oct 4. doi: 10.5414/CP204037. Online ahead of print, 2021.**

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IMSUT Hospital

Department of AIDS Vaccine Development
エイズワクチン開発担当

Professor	Tetsuro Matano, M.D., D.M.Sc.		教授（委嘱）	博士（医学）	俣 野 哲 朗
Visiting Associate Professor	Ai Kawana-Tachikawa, D.M.Sc.		客員准教授	博士（医学）	立川（川名） 愛

We are working on Microbiology and Immunology to elucidate the immune mechanism for retroviral control in vivo. In particular, we are studying virus-host immune interaction and viral evolution using non-human primate models and human clinical samples derived from African and Asian countries as well as Japan. Furthermore, we are developing vaccines eliciting antibody and/or cytotoxic T lymphocyte responses targeting pathogens including HIV-1, HTLV-1, and SARS-CoV-2.

1. A potent anti-simian immunodeficiency virus neutralizing antibody induction associated with a germline immunoglobulin gene polymorphism in rhesus macaques.

Saori Matsuoka[1], Takeo Kuwata[2], Hiroshi Ishii[1], Tsuyoshi Sekizuka[3], Makoto Kuroda[3], Masato Sano[1], Midori Okazaki[1], Hiroyuki Yamamoto[1], Mikiko Shimizu[2], Shuzo Matsushita[2], Yohei Seki[4], Akatsuki Saito[4], Hiromi Sakawaki[5], Vanessa M. Hirsch[6], Tomoyuki Miura[5], Hirofumi Akari[4], Tetsuro Matano: [1]AIDS Research Center, National Institute of Infectious Diseases, Tokyo, Japan; [2]Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto, Japan; [3]Pathogen Genomics Center, National Institute of Infectious Disease, Tokyo, Japan; [4]Primate Research Institute, Kyoto University, Inuyama, Japan; [5]Institute for Frontier Life and Medical Sciences, Kyoto University, Kyoto, Japan; [6]Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA

Virus infection induces B cells with a wide variety of BCR repertoires. Patterns of induced BCR repertoires are different in individuals, while the underlying mechanism causing this difference remains largely unclear. In particular, the impact of germline BCR Ig gene polymorphism on B cell/antibody induction has not fully been determined. In this study, we found a potent antibody induction associated with a germline BCR Ig gene polymorphism. B404-class antibodies, which were previously reported as potent anti-SIV neutralizing antibodies using the germline VH3.33 gene-derived Ig heavy chain, were induced in five of ten rhesus macaques after SIVsmH635FC infection. Investigation of VH3.33 genes in B404-class antibody inducers and non-inducers revealed association of B404-class antibody induction with a germline VH3.33 polymorphism. Analysis of reconstructed antibodies indicated that the VH3.33 residue 38 is the determinant for B404-class antibody induction. B404-class antibodies were induced in all the macaques possessing the B404-associated VH3.33 allele, even under undetectable viremia. Our results show that a single nucleotide polymorphism in germline VH genes could be a determinant for induction of potent antibodies against virus infection, implying that germline VH-gene polymorphisms can be a factor restricting effective antibody induction or responsiveness to vaccination.

2. Association of *Human Leukocyte Antigen DRB1*09:01* with severe COVID-19.

Alitzel Anzurez[1], Izumi Naka[7], Shoji Miki[1], Kaori Hosoya-Nakayama[1], Mariko Isshiki[7], Yusuke Watanabe[7], Midori Nakamura-Hoshi[1], Sayuri Seki[1], Takayuki Matsumura[8], Tomohiro Takano[8], Taishi Onodera[8], Yu Adachi[8], Saya Moriyama[8], Kazutaka Terahara[8], Natsuo Tachikawa[9], Yoshihiro Yoshimura[9], Hiroaki Sasaki[9], Hiroshi Horiuchi[9], Nobuyuki Miyata[9], Kazuhito Miyazaki[9], Michiko Koga[10], Kazuhiko Ikeuchi[10], Hiroyuki Nagai[10], Makoto Saito[10], Eisuke Adachi[10], Hiroshi Yotsuyanagi[10], Satoshi Kutsuna[11], Akira Kawashima[11], Yusuke Miyazato[11], Noriko Kinoshita[11], Chiyoko Kouno[12], Kensuke Tanaka[12], Yoshimasa Takahashi[8], Tadaki Suzuki[13], Tetsuro Matano, Jun Ohashi[7], Ai Kawana-Tachikawa: [7]Department of Biological Sciences, Graduate School of Science, University of Tokyo, Tokyo, Japan; [8]Department of Immunology, National Institute of Infectious Diseases, Tokyo, Japan; [9]Department of Infectious Diseases, Yokohama Municipal Citizens' Hospital, Kanagawa, Japan; [10]Division of Infectious Diseases, Advanced Clinical Research Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan; [11]Disease Control and Prevention Center, National Center for Global Health and Medicine, Tokyo, Japan; [12]JR Tokyo General Hospital, Tokyo, Japan; [13]Department of Pathology, National Institute of Infectious Diseases, Tokyo, Japan

HLA-A, -C, -B, and -DRB1 genotypes were analyzed in Japanese COVID-19 patients to investigate the association of HLA with severe COVID-19. Analysis of 32 common HLA alleles at four loci revealed a significant association between HLA-DRB1*09:01 and severe COVID-19 when age, sex, and other common HLA alleles at the DRB1 locus were adjusted. The DRB1*09:01 allele was more significantly associated with risk for severe COVID-19 compared to preexisting medical conditions such as hypertension, diabetes, and cardiovascular diseases. These results indicate a potential role for HLA in predisposition to severe COVID-19.

3. Dysbiotic fecal microbiome in HIV-1 infected individuals in Ghana.

Prince Kofi Parbie[1], Taketoshi Mizutani[14], Aya Ishizaka[14], Ai Kawana-Tachikawa, Lucky Ronald Runtuwene[1], Sayuri Seki[1], Christopher Zaab-Yen Abana[15], Dennis Kushitor[15], Evelyn Yayra Bonney[15], Sampson Badu Ofori[16], Satoshi Uematsu[14], Seiya Imoto[14], Yasumasa Kimura[14], Hiroshi Kiyono[14], Koichi Ishikawa[1], William Kwabena Ampofo[15], Tetsuro Matano: [14]International Research and Development Center for Mucosal Vaccines, Institute of Medical Science, University of Tokyo, Tokyo, Japan; [15]Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana; [16]Regional Hospital Koforidua, Ghana Health Service, Koforidua, Ghana

HIV-1 infected individuals under antiretroviral therapy can control viremia but often develop non-AIDS diseases such as cardiovascular and metabolic disorders. Gut microbiome dysbiosis has been indicated to be associated with progression of these diseases. Analyses of gut/fecal microbiome in individual regions are important for our understanding of pathogenesis in HIV-1 infections. However, data on gut/fecal microbiome has not yet been accumulated in West Africa. In this study, we examined fecal microbiome compositions in HIV-1 infected adults in Ghana, where approximately two-thirds of infected adults are females. In a cross-sectional case-control study, age- and gender-matched HIV-1 infected adults and seronegative controls were enrolled. Alpha diversity of fecal microbiome in HIV + was significantly reduced compared to HIV- and associated with CD4 counts. HIV + showed reduction in varieties of bacteria including *Faecalibacterium*, the most abundant in seronegative controls, but enrichment of *Proteobacteria*. Ghanaian HIV + exhibited enrichment of *Dorea* and *Blautia*; bacteria groups whose depletion has been reported in HIV-1 infected individuals in several other cohorts. Furthermore, HIV + in our cohort exhibited a depletion of *Prevotella*, a genus whose enrichment has recently been shown in MSM regardless of HIV-1 status. This study revealed the characteristics of dysbiotic fecal microbiome in HIV-1 infected adults in Ghana, a representative of West African populations.

4. Phylodynamic analysis reveals changing transmission dynamics of HIV-1 CRF01_AE in Japan from heterosexuals to men who have sex with men.

Machiko Otani, Teiichiro Shiino[1], Makiko Kondo[17], Atsuko Hachiya[18], Masako Nishizawa[1], Tadashi Kikuchi[1], Tetsuro Matano, Japanese Drug Resistance HIV-1 Surveillance Network: [17]Division of Microbiology, Kanagawa Prefectural Institute of Public Health, Kanagawa, Japan; [18]Clinical Research Centre, National Hospital Organization Nagoya Medical Centre, Aichi, Japan

HIV-1 CRF01_AE is the second major subtype in Japan. Our previous study indicated that CRF01_AE was predominantly circulating in heterosexuals/injecting drug users (IDUs). With implications of increased CRF01_AE infections among men who have sex with men (MSM), this study sought to investigate whether transmission dynamics of CRF01_AE infections in Japan has changed. Sequences from 8,032 newly diagnosed HIV-1-infected individuals were analysed. The individuals were predominantly Japanese (64%) and male (72%). MSM became the predominant transmission risk from 2014. We identified 30 transmission clusters (TCs) and 48 pairs including 40% of individuals. MSM were approximately 5 times

more likely to be in a TC compared to heterosexuals and were the major contributors to TCs. tMRCA suggests that MSM TCs emerged from 1996 and became predominant around 2000. CRF01_AE spread among MSM with frequent and continuous cluster formations and became predominant transmission risk. This study suggests that CRF01_AE transmission has shifted from heterosexuals/IDUs to MSM.

5. Neutralizing antibody induction associated with a germline immunoglobulin gene polymorphism in neutralization-resistant SIVsmE 543-3 infection.

Yuto Nomura, Saori Matsuoka[1], Midori Okazaki[1], Takeo Kuwata[2], Tetsuro Matano, Hiroshi Ishii[1]

Antibody responses are crucial for the control of virus infection. Understanding of the mechanism of antibody induction is important for the development of a vaccine eliciting effective anti-virus antibodies. Virus-specific BCR/antibody repertoires are different among individuals, but determinants for this difference remains largely unclear. We have recently reported that a germline BCR IgG gene polymorphism (VH3.33_ET or VH3.33_VI) in rhesus macaques is the determinant for induction of potent B404-class anti-SIV neutralizing antibodies (NAbs) in NAb-sensitive SIVsmH635FC infection. In this study, we examined whether NAb-resistant SIVsmE543-3 infection can induce the anti-SIV NAbs associated with the germline VH3.33 polymorphism. Anti-SIVsmE543-3 NAbs were induced in all the macaques possessing the VH3.33_ET allele but not in those without VH3.33_ET in the chronic phase of SIVsmE543-3 infection. Next generation sequencing analysis of BCR VH genes found B404-class antibody sequences only in those with VH3.33_ET. These results indicate that anti-SIVsmE543-3 NAb induction associated with the germline BCR IgG gene polymorphism can be triggered by infection with NAb-resistant SIVsmE543-3. This animal model would be useful for elucidation of the mechanism of potent antibody induction against NAb-resistant viruses.

6. Subacute SARS-CoV-2 replication can be controlled in the absence of CD8$^+$ T cells in cynomolgus macaques.

Takushi Nomura[1], Hiroyuki Yamamoto[1], Masako Nishizawa[1], Trang Thi Thu Hau[1], Shigeyoshi Harada[1], Hiroshi Ishii[1], Sayuri Seki[1], Midori Nakamura-Hoshi[1], Midori Okazaki[1], Sachie Daigen[1], Ai Kawana-Tachikawa, Noriyo Nagata[13], Naoko Iwata-Yoshikawa[13], Nozomi Shiwa[13], Shun Iida[13], Harutaka Katano[13], Tadaki Suzuki[13], Eun-Sil Park[19], Ken Maeda[19], Yuriko Suzaki[20], Yasushi Ami[20], Tetsuro Matano: [19]Department of Veterinary Science, National Institute of Infectious Diseases, Tokyo, Japan; [20]Division of Laboratory Animal Research, National Institute of Infectious Diseases, Tokyo, Japan

SARS-CoV-2 infection presents a wide spectrum of clinical manifestations ranging from asymptomatic to fatal respiratory failure. The determinants for failure in viral control and/or fatal disease progression have not been elucidated fully. Both acquired immune effectors, antibodies and CD8$^+$ T cells, are considered to contribute to viral control. However, it remains unknown whether a deficiency in either of these two arms is directly linked to failure in the control of SARS-CoV-2 replication. In this study, to know the requirement of CD8$^+$ T cells for viral control after the establishment of infection, we examined the effect of CD8$^+$ cell depletion by monoclonal anti-CD8 antibody administration in the subacute phase on SARS-CoV-2 replication in cynomolgus macaques. Unexpectedly, our analysis revealed no significant impact of CD8$^+$ cell depletion on viral replication, indicating that subacute SARS-CoV-2 replication can be controlled in the absence of CD8$^+$ T cells. CD8$^+$ T-cell responses may contribute to viral control in SARS-CoV-2 infection, but this study suggests that CD8$^+$ T-cell dysfunction may not solely lead to viral control failure or fatal disease progression.

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IMSUT Distinguished Professor Unit

Division of Stem Cell Therapy
幹細胞治療部門

Project Professor	Hiromitsu Nakauchi, M.D., Ph.D	特任教授　医学博士	中　内　啓　光
Project Assistant Professor	Hideki Masaki, Ph.D.	特任助教　博士（理学）	正　木　英　樹

We are working on uncovering new diseases, elucidating the causes of disease, and developing therapeutic modalities by connecting the knowledge and methodology of basic science including immunology, molecular biology, cell biology, and developmental engineering with clinical medicine. Our ultimate goal is to contribute to establishing new frontiers of stem cell therapy and to make clinical applications of stem cells a reality.

1. Cas9-AAV6 gene correction of beta-globin in autologous HSCs improves sickle cell disease erythropoiesis in mice.

Adam C Wilkinson[1 2], Daniel P Dever[3 4], Ron Baik[3 4], Joab Camarena[3 4], Ian Hsu[3 5], Carsten T Charlesworth[3 5], Chika Morita[3 5], Hiromitsu Nakauchi[6 7 8], Matthew H Porteus[9 10]

[1]Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Lorry I. Lokey Stem Cell Research Building, 265 Campus Drive, Stanford, CA, USA.
[2]Department of Genetics, Stanford University School of Medicine, Stanford, CA, USA.
[3]Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Lorry I. Lokey Stem Cell Research Building, 265 Campus Drive, Stanford, CA, USA.
[4]Department of Pediatrics, Stanford University School of Medicine, Stanford, CA, USA.
[5]Department of Genetics, Stanford University School of Medicine, Stanford, CA, USA.
[6]Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Lorry I. Lokey Stem Cell Research Building, 265 Campus Drive, Stanford, CA, USA.
[7]Department of Genetics, Stanford University School of Medicine, Stanford, CA, USA.
[8]Division of Stem Cell Therapy, Distinguished Professor Unit, The Institute of Medical Science, The University of Tokyo
[9]Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Lorry I. Lokey Stem Cell Research Building, 265 Campus Drive, Stanford, CA, USA.
[10]Department of Pediatrics, Stanford University School of Medicine, Stanford, CA, USA.

CRISPR/Cas9-mediated beta-globin (HBB) gene correction of sickle cell disease (SCD) patient-derived hematopoietic stem cells (HSCs) in combination with autologous transplantation represents a recent paradigm in gene therapy. Although several Cas9-based HBB-correction approaches have been proposed, functional correction of in vivo erythropoiesis has not been investigated previously. Here, we use a humanized globin-cluster SCD mouse model to study Cas9-AAV6-mediated HBB-correction in functional HSCs within the context of autologous transplantation. We discover that long-term multipotent HSCs can be gene corrected ex vivo and stable hemoglobin-A production can be achieved in vivo from HBB-corrected HSCs following autologous transplantation. We observe a direct correlation between increased HBB-corrected myeloid chimerism and

normalized in vivo red blood cell (RBC) features, but even low levels of chimerism resulted in robust hemoglobin-A levels. Moreover, this study offers a platform for gene editing of mouse HSCs for both basic and translational research.

2. Generation of Functional Organs Using a Cell-Competitive Niche in Intra-and Inter-species Rodent Chimeras.

Toshiya Nishimura [1], Fabian P Suchy [2], Joydeep Bhadury [3], Kyomi J Igarashi [4], Carsten T Charlesworth [2], Hiromitsu Nakauchi [5]

Division of Stem Cell Therapy, Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan; Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford, CA 94305, USA.
[2]Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford, CA 94305, USA.
[3]Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford, CA 94305, USA; Dept of Laboratory Medicine, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, SE 413 45, Gothenburg, Sweden.
[4]Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford, CA 94305, USA; Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305, USA.
[5]Division of Stem Cell Therapy, Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan; Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford, CA 94305, USA; Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305, USA.

Interspecies organ generation via blastocyst complementation has succeeded in rodents, but not yet in evolutionally more distant species. Early developmental arrest hinders the formation of highly chimeric fetuses. We demonstrate that the deletion of insulin-like growth factor 1 receptor (Igf1r) in mouse embryos creates a permissive "cell-competitive niche" in several organs, significantly augmenting both mouse intraspecies and mouse/rat interspecies donor chimerism that continuously increases from embryonic day 11 onward, sometimes even taking over entire organs within intraspecies chimeras. Since Igf1r deletion allows the evasion of early developmental arrest, interspecies fetuses with high levels of organ chimerism can be generated via blastocyst complementation. This observation should facilitate donor cell contribution to host tissues, resulting in whole-organ generation via blastocyst complementation across wide evolutionary distances.

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IMSUT Distinguished Professor Unit

Division of Mucosal Immunology
粘膜免疫学部門

Project Professor	Hiroshi Kiyono, D.D.S., Ph.D.	特任教授	医学博士	清　野　　宏
Project Associate Professor	Yosuke Kurashima, Ph.D.	特任准教授	博士(医学)	倉　島　洋　介
Project Senior Assistant Professor	Rika Nakahashi, Ph.D.	特任講師	博士(医学)	中　橋　理　佳

The mucosal immune system not only senses pathogenic antigens such as pathogens and allergens, but also establishes tolerance that does not react excessively to beneficial antigens such as food-derived proteins and commensal microorganisms. Our laboratory's mission is to elucidate and understand the uniqueness of the mucosal immune system which controls the immunological balancing act between the elimination of and commensalism with harmful and beneficial antigens, respectively, and aim to develop the basic platform for creating the novel strategies of prevention and treatment of various infectious and immunological diseases by the fusion science with agriculture, engineering and plant biology.

1. Development of Nanogel-based nasal vaccine against RSV infection.

Shingo Umemoto[1, 2], Rika Nakahashi[1], Yohei Uchida[1], Shiho Kurokawa[1], Yoshikazu Yuki[1], and Hiroshi Kiyono[1, 3-5]

[1] Department of Mucosal Immunology, IMSUT Distinguished Professor Unit, The Institute of Medical Science, The University of Tokyo, [2] Department of Otolaryngology Head and Neck Surgery, Faculty of Medicine, Oita University, [3] International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo, [4] Division of Gastroenterology, Department of Medicine, CU-UCSD Center for Mucosal Immunology, Allergy and Vaccines, University of California, [5] Mucosal Immunology and Allergy Therapeutics, Institute for Global Prominent Research, Chiba University.

Respiratory syncytial virus (RSV) is a leading cause of upper and lower respiratory infections in children younger than five and elderly people. Although a prophylactic vaccine has been desired for many years, it has not yet reached clinical application.

We have developed a novel nasal vaccine against RSV, targeting an ectodomain of RSV and using a cationic cholesteryl-group-bearing pullulan (cCHP)-nanogel as a vaccine delivery system. Furthermore, we have previously revealed the protective effects of this vaccine against RSV in both upper and lower respiratory tracts of mice. We also demonstrated that the antibodies induced by this nasal vaccine do not have a direct neutralizing effect, but eliminate the virus via antibody dependent cellular cytotoxicity (ADCC).

This year, we revealed the RSV (ectodomain protein-) specific antibodies in serum and nasal wash from immunized mice induced by the nasal vaccine directly bind to RSV using flow cytometry. Although we have demonstrated that intranasally immunized mice showed higher viral suppression in the upper respiratory tract compared to mice immunized with the same antigen intraperitoneally, the mechanism of viral elimination in the upper respiratory tract remained unclear. In the current study, it was shown that IgA in nasal wash effectively binds RSV, which may lead to the elucidation of the virus elimination mechanism in the upper respiratory tract.

We have also analyzed the protective mechanisms of this novel nasal vaccine against RSV in cotton rats, which are susceptible to a surprisingly large variety of human pathogens. Our data showed that the nasal vaccine induced both RSV (ectodomain protein)-specific serum IgG and mucosal secretory IgA, similar to that seen in mice. And we also have results showing that nasal immunization with this vaccine reduced RSV replication more both in the upper and lower respiratory tract of cotton rats than in those of unimmunized cotton rats. The results that sufficient protection against RSV was demonstrated not only in mice but also in cotton rats is important for consideration of future clinical applications.

These results provide promising evidence for the protective efficacy and safety of this novel nasal vaccine against RSV.

2. Intestinal immune signals regulate Paneth cell granule formation and α-defensin secretion

Mariko Kamioka[1–4], Yoshiyuki Goto[2,5], Kiminori Nakamura[6], Yuki Yokoi[6], Rina Sugimoto[6], Shuya Ohira[6], Yosuke Kurashima[1–4,7], Shingo Umemoto[1,3,8], Shintaro Sato[1,9,10], Jun Kunisawa[2,4], Yu Takahashi[11], Steven E. Domino[12], Jean-Christophe Renauld[13], Susumu Nakae[14], Yoichiro Iwakura[15], Peter B. Ernst[3, 16-18], Tokiyoshi Ayabe[6] and Hiroshi Kiyono[1–3,18*]

[1] Department of Mucosal Immunology, IMSUT Distinguished Professor Unit, The Institute of Medical Science, The University of Tokyo, [2] International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo, [3] Department of Medicine, School of Medicine and Chiba University-UCSD Center for Mucosal Immunology, Allergy and Vaccine (CU-UCSD cMAV), University of California San Diego, [4] Laboratory of Vaccine Materials, Center for Vaccine and Adjuvant Research and Laboratory of Gut Environmental System, National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN), [5] Division of Molecular Immunology, Medical Mycology Research Center, Chiba University, [6] Department of Cell Biological Science, Graduate School of Life Science, Faculty of Advanced Life Science, Hokkaido University, [7] Department of Innovative Medicine, Graduate School of Medicine, Chiba University, [8] Department of Otolaryngology & Head and Neck Surgery, Faculty of Medicine, Oita University, [9] Mucosal Vaccine Project, BIKEN Innovative Vaccine Research Alliance Laboratories, Research Institute for Microbial Diseases, Osaka University, [10] Department of Immunology and Genomics, Osaka City University Graduate School of Medicine, [11] Food Biochemistry Laboratory, Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, [12] Department of Obstetrics and Gynecology, Cellular and Molecular Biology Program, University of Michigan Medical Center, [13] Ludwig Institute for Cancer Research and Université Catholique de Louvain, [14] Graduate School of Integrated Sciences for Life, Hiroshima University, [15] Center for Experimental Animal Models, Institute for Biomedical Sciences, Tokyo University of Science, [16] Division of Comparative Pathology and Medicine, Department of Pathology, University of California San Diego, [17] Center for Veterinary Sciences and Comparative Medicine, University of California, [18] Future Medicine Education and Research Organization, Department of Immunology, Graduate School of Medicine, Chiba University.

The gastrointestinal tract is constantly exposed to numerous foreign antigens. Intestinal epithelial cell layer acts as a first line of defense and is divided into villi and crypt regions. In the crypts, epithelial stem cells and Paneth cells are preferentially located. Paneth cells release granules containing a variety of antimicrobial peptides as a major part of the host innate immune system. α-defensin is most abundant and highly bactericidal peptide specifically produced by Paneth cells.

It has been known that crypts are surrounded by immune cells. Type3 innate lymphoid cells located beneath of crypts preferentially produce Interleukin 22 (IL-22) known as innate immune signaling. We found that IL-22 promotes the differentiation of Paneth cells with matured granules containing α-defensin. We further found that IL-17a also regulates the amount of α-defensin in the intestinal lumen.

Our results indicate that the cell fate and function of Paneth cells are regulated by multiple immune signals for the production and secretion of α-defensin in gastrointestinal tract. α-defensin plays a crucial role for the creation and maintenance of intestinal homeostasis, thus we concluded that the mutual interaction of Paneth cells and immune cells provide healthy intestinal environment.

3. Development of antibody-fragment-producing rice for norovirus gastroenteritis

Ai Sasou[1], Yoshikazu Yuki[1], Shiho Kurokawa[1], Shintaro Sato[2,3], Yuki Goda[1], Masao Uchida[4], Naomi Matsumoto[2], Hiroshi Sagara[5], Yuji Watanabe[5], Masaharu Kuroda[6], Naomi Sakon[7], Kotomi Sugiura[1], Rika Nakahashi[1,8], Hiroshi Ushijima[9], Kohtaro Fujihashi[10,11], Hiroshi Kiyono[8,12,13]

[1] Division of Mucosal Vaccines, International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo, [2] Mucosal Vaccine Project, BIKEN Innovative Vaccine Research Alliance Laboratories, Research Institute for Microbial Diseases, Osaka University, [3] Department of Immunology and Genomics, Graduate School of Medicine, Osaka City University, [4] Drug Discovery Research, Astellas

Pharma Inc., [5] Medical Proteomics Laboratory, The Institute of Medical Science, University of Tokyo, [6] The National Agriculture and Food Research Organization, [7] Department of Microbiology, Osaka Institute of Public Health, [8] Division of Mucosal Immunology, IMSUT Distinguished Professor Unit, The Institute of Medical Science, The University of Tokyo, [9] Department of Pathology and Microbiology, Nihon University School of Medicine, [10] Division of Clinical Vaccinology, International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo, [11] Department of Pediatric Dentistry, The University of Alabama at Birmingham, [12] Department of Immunology, Graduate School of Medicine, Chiba University, [13] Division of Gastroenterology, Department of Medicine, Chiba University – University of California San Diego Center for Mucosal Immunology, Allergy, and Vaccine, University of California, San Diego.

Human norovirus can cause acute nonbacterial gastroenteritis on people of any age, and can be more serious for the elderly, infants, and people who are immunocompromised. However, there is currently no approved vaccine or treatment for norovirus infection. Based on this background, we have developed MucoRice-VHH which produce a variable domain of a llama heavy chain antibody fragment (VHH) specific for human norovirus as a neutralizing antibody by using a rice transgenic system as a novel treatment against norovirus gastroenteritis. VHH is a small protein that is stable to heat and acid and resembles a monoclonal antibody, making it an attractive and useful antibody for oral immunotherapy against intestinal infections.

Indeed, an evaluation by using a human norovirus growth system with human induced pluripotent stem cell-derived intestinal epithelial cells (IECs) revealed that MucoRice-derived VHH showed high neutralizing activity against norovirus. It was also demonstrated that MucoRice-VHH retained its neutralizing activity even after heat treatment at 90°C for 20 minutes. These results suggested that the MucoRice-VHH could be a novel oral immunotherapy candidate for norovirus infections, and also lead to the development of prophylactic vaccine for healthy adults, children, elderly, and immunocompromised patients.

4. Cytochrome c released from intra-tissue opportunistic bacteria induce apoptosis of host cells.

Naoko Shibata[1,2], Jun Kunisawa[2-6*], Masahiro Ando[6], Masato Hosokawa[7], Shumpei Horii[1,8], Koji Hosomi[3], Haruko Takeyama[1,6,7,8], and Hiroshi Kiyono[2,9,10,*]

[1] Graduate School of Advanced Science and Engineering, Waseda Univ. [2] International Research and Development Center for Mucosal Vaccines, Univ. of Tokyo. [3] Laboratory of Vaccine Materials, Center for Vaccine and Adjuvant Research, and Laboratory of Gut Environmental System, National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN). [4] Graduate School of Medicine, Graduate School of Pharmaceutical Sciences, Graduate School of Density, Osaka Univ. [5] Graduate School of Medicine, Kobe Univ. [6] Research Organization for Nano and Life Innovation, Waseda Univ. [7] Institute for Advanced Research of Biosystem Dynamics, Waseda Univ. [8] Computational Bio Big-Data Open Innovation Laboratory, Advanced Industrial Science and Technology, Waseda Univ. [9] Department of Medicine, Univ. of California [10] Graduate School of Medicine, Chiba Univ.

Our intestinal tracts are continuously exposed to various antigens, including dietary food materials, commensal bacteria, and pathogenic bacteria. The mucosal immune systems developed for the elimination of pathogenic bacteria and tolerance to dietary antigens and symbiotic bacteria. On the other hand, the deteriorations of bacterial compositions cause gut-related immune diseases such as food allergies and inflammatory bowel diseases. Therefore, it is becoming more critical to understand the interactions between intestinal bacteria and the mucosal immune system in both steady- and symbiotically disrupted states. Peyer's patches (PPs) are one of the primary gut-associated lymphoid tissues in the small intestine.

We previously identified *Alcaligenes* as a symbiont within dendritic cells (DCs) in the Peyer's patches. *Alcaligenes* contributes to IgA production via the appropriate activation of DCs and the impaired symbiosis induces inflammatory responses; however, it remains to be investigated how the symbiotic relationship is maintained. Here, we show that *Alcaligenes* changed the morphology from rod- to filament-shape, which was induced via quorum-sensing-dependent manner. The changes associated with the increase and release of cytochrome c, an apoptosis-inducing factor against eukaryotes, and consistently induced the apoptosis of host DCs. We also identified that filament-shape *Alcaligenes* produce membrane vesicles known as bacterial signal transporters. We found that short-chain fatty acids are enriched in the membrane vesicles of filament-shape *Alcaligenes*. Although short-chain fatty acids are typical bioactive molecules derived from intestinal bacteria, no reports indicate that they are encapsulated in membrane vesicles. These findings indicated that the functional changes of *Alcaligenes* associated with their morphology can be a regulator of symbiotic communication between *Alcaligenes* and DCs.

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IMSUT Distinguished Professor Unit

Division of Virology
ウイルス感染部門

Project Professor	Yoshihiro Kawaoka, D.V.M., Ph.D.	特任教授	獣医学博士	河 岡 義 裕
Project Assistant Professor	Kiyoko Iwatsuki-Horimoto, D.V.M., Ph.D.	特任助教	博士(獣医学)	岩 附 研 子
Project Assistant Professor	Maki Kiso, D.V.M., Ph.D.	特任助教	博士(医学)	木 曽 真 紀

Viruses can cause devastating diseases. The long-term goal of our research is to understand the molecular pathogenesis of viral diseases by using influenza virus, Ebola virus, and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections as models. Interactions between viral and host gene products during viral replication determine the consequences of infection (i.e., the characteristics of disease manifestation, whether limited or widespread); hence, our research has centered on such interactions during these viral infections.

1. Protective Immunity and Persistent Lung Sequalae in Domestic Cats after SARS-CoV-2 Infection

Chiba S[1], Halfmann PJ[1], Hatta M[1], Maemura T[1], Fan S[1], Armbrust T[1], Swartley OM[1], Crawford LK[1], Kawaoka Y: [1]Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, USA.

SARS-CoV-2 readily transmits between domestic cats. We found that domestic cats that recover from an initial infection might be protected from reinfection. However, we also found long-term persistence of inflammation and other lung lesions after infection in cats, despite a lack of clinical symptoms and limited viral replication in the lungs.

2. Plasticity of the Influenza Virus H5 HA Protein

Kong H[1], Burke DF[2], da Silva Lopes TJ[1], Takada K, Imai M, Zhong G[1], Hatta M[1], Fan S[1], Chiba S[1], Smith D[2], Neumann G[1], Kawaoka Y: [2]Department of Zoology, University of Cambridge, Cambridge, United Kingdom.

Since the emergence of highly pathogenic avian influenza viruses of the H5 subtype, the major viral antigen, hemagglutinin (HA), has undergone constant evolution, resulting in numerous genetic and antigenic (sub)clades. To explore the consequences of amino acid changes at sites that may affect the antigenicity of H5 viruses, we simultaneously mutated 17 amino acid positions of an H5 HA by using a synthetic gene library that, theoretically, encodes all combinations of the 20 amino acids at the 17 positions. All 251 mutant viruses sequenced possessed ≥13 amino acid substitutions in HA, demonstrating that the targeted sites can accommodate a substantial number of mutations. Selection with ferret sera raised against H5 viruses of different clades resulted in the isolation of 39 genotypes. Further analysis of seven variants demonstrated that they were antigenically different from the parental virus and replicated efficiently in mammalian cells. Our data demonstrate the substantial plasticity of the influenza virus H5 HA protein, which may lead to novel antigenic variants.

3. Antibody titers against SARS-CoV-2 decline, but do not disappear for several months

Yamayoshi S, Yasuhara A, Ito M, Akasaka O[3], Nakamura M[4], Nakachi I[5], Koga M[6,7], Mitamura K[8], Yagi K[9], Maeda K[10], Kato H[11,12], Nojima M[13], Pattinson

D[1], Ogura T[14], Baba R[5], Fujita K[14], Nagai H[7], Yamamoto S, Saito M[6,7], Adachi E[7], Ochi J[15], Hattori SI[10], Suzuki T[16], Miyazato Y[16], Chiba S[1], Okuda M, Murakami J, Hamabata T, Iwatsuki-Horimoto K, Nakajima H[12], Mitsuya H[10], Omagari N[16], Sugaya N[17], Yotsuyanagi H[6,7], Kawaoka Y: [3]Emergency Medical Center, Fujisawa City Hospital, Japan. [4]Department of Pulmonary Medicine, Tokyo Saiseikai Central Hospital, Japan. [5]Pulmonary division, Department of Internal Medicine, Saiseikai Utsunomiya Hospital, Japan. [6]Division of Infectious Diseases, Advanced Clinical Research Center, Institute of Medical Science, University of Tokyo, Japan. [7]Department of Infectious Diseases and Applied Immunology, IMSUT Hospital of Institute of Medical Science, the University of Tokyo, Japan. [8]Division of Infection Control, Eiju General Hospital, Japan [9]Department of Pulmonary Medicine, Department of Medicine, Keiyu Hospital, Japan. [10]Department of Refractory Viral Infections, National Center for Global Health and Medicine Research Institute, Japan. [11]Infection Prevention and Control Department, Yokohama City University Hospital, Japan. [12]Department of Hematology and Clinical Immunology, Yokohama City University School of Medicine, Japan. [13]Center for Translational Research, Institute of Medical Science Hospital, University of Tokyo, Japan. [14]Department of Emergency Medicine and Critical Care Medicine, Saiseikai Utsunomiya Hospital, Japan. [15]Department of Respiratory Medicine, Eiju General Hospital, Japan. [16]Disease Control and Prevention Center, National Center for Global Health and Medicine Hospital, Japan. [17]Department of Pediatrics, Keiyu Hospital, Japan.

To develop an effective vaccine against a novel viral pathogen, it is important to understand the longitudinal antibody responses against its first infection. Here we performed a longitudinal study of antibody responses against SARS-CoV-2 in symptomatic patients. Sequential blood samples were collected from 39 individuals at various timepoints between 0 and 154 days after onset. IgG or IgM titers to the receptor binding domain (RBD) of the S protein, the ectodomain of the S protein, and the N protein were determined by using an ELISA. Neutralizing antibody titers were measured by using a plaque reduction assay. The IgG titers to the RBD of the S protein, the ectodomain of the S protein, and the N protein peaked at about 20 days after onset, gradually decreased thereafter, and were maintained for several months after onset. Extrapolation modeling analysis suggested that the IgG antibodies were maintained for this amount of time because the rate of reduction slowed after 30 days post-onset. IgM titers to the RBD decreased rapidly and disappeared in some individuals after 90 days post-onset. All patients, except one, possessed neutralizing antibodies against authentic SARS-CoV-2, which they retained at 90 days after onset. The highest antibody titers in patients with severe infections were higher than those in patients with mild or moderate infections, but the decrease in antibody titer in the severe infection cohort was more remarkable than that in the mild or moderate infection cohort. Although the number of patients is limited, our results show that the antibody response against the first SARS-CoV-2 infection in symptomatic patients is typical of that observed in an acute viral infection.

4. Increased risk of rhinovirus infection in children during the coronavirus disease-19 pandemic.

Takashita E[18], Kawakami C[19], Momoki T[19], Saikusa M[19], Shimizu K[19], Ozawa H[19], Kumazaki M[19], Usuku S[19], Tanaka N[19], Okubo I[19], Morita H[18], Nagata S[18], Watanabe S[18], Hasegawa H[18], Kawaoka Y: [18]Influenza Virus Research Center, National Institute of Infectious Diseases, Tokyo, Japan. [19]Yokohama City Institute of Public Health, Kanagawa, Japan.

Coronavirus disease (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first detected in Japan in January 2020 and has spread throughout the country. Previous studies have reported that viral interference among influenza virus, rhinovirus, and other respiratory viruses can affect viral infections at the host and population level. To investigate the impact of COVID-19 on influenza and other respiratory virus infections, we analyzed clinical specimens collected from 2244 patients in Japan with respiratory diseases between January 2018 and September 2020. The frequency of influenza and other respiratory viruses (coxsackievirus A and B; echovirus; enterovirus; human coronavirus 229E, HKU1, NL63, and OC43; human metapneumovirus; human parainfluenza virus 1, 2, 3, and 4; human parechovirus; human respiratory syncytial virus; human adenovirus; human bocavirus; human parvovirus B19; herpes simplex virus type 1; and varicella-zoster virus) was appreciably reduced among all patients during the COVID-19 pandemic except for that of rhinovirus in children younger than 10 years, which was appreciably increased. COVID-19 has not spread among this age group, suggesting an increased risk of rhinovirus infection in children. Rhinovirus infections should be continuously monitored to understand their increased risk during the COVID-19 pandemic and viral interference with SARS-CoV-2.

5. Characterization of a new SARS-CoV-2 variant that emerged in Brazil.

Imai M, Halfmann PJ[1], Yamayoshi S, Iwatsuki-Horimoto K, Chiba S[1], Watanabe T[20], Nakajima N[21], Ito M, Kuroda M[1], Kiso M, Maemura T[1], Takahashi

K[21], Loeber S[22], Hatta M[1], Koga M[6,7], Nagai H[7], Yamamoto S, Saito M[6,7], Adachi E[7], Akasaka O[3], Nakamura M[4], Nakachi I[5], Ogura T[14], Baba R[5], Fujita K[14], Ochi J[15], Mitamura K[8], Kato H[11,12], Nakajima H[12], Yagi K[9], Hattori SI[10], Maeda K[10], Suzuki T[16], Miyazato Y[16], Valdez R[23], Gherasim C[23], Furusawa Y, Okuda M, Ujie M, Lopes TJS[1], Yasuhara A, Ueki H, Sakai-Tagawa Y, Eisfeld AJ[1], Baczenas JJ[24,25], Baker DA[24], O'Connor SL[24,25], O'Connor DH[24,25], Fukushi S[26], Fujimoto T[27], Kuroda Y[28], Gordon A[29], Maeda K[28], Ohmagari N[16], Sugaya N[17], Yotsuyanagi H[6,7], Mitsuya H[10], Suzuki T[21], Kawaoka Y: [20]Department of Molecular Virology, Research Institute for Microbial Diseases, Osaka University, Osaka 565-0871, Japan. [21]Department of Pathology, National Institute of Infectious Diseases, Tokyo 162-8640, Japan. [22]Department of Surgical Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI 53706. [23]Department of Pathology, University of Michigan, Ann Arbor, MI 48109. [24]Department of Pathology and Laboratory Medicine, University of Wisconsin-Madison, Madison, WI 53705. [25]Wisconsin National Primate Research Center, University of Wisconsin-Madison, Madison, WI 53715. [26]Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, MI 48109. [27]Center for Emergency Preparedness and Response, National Institute of Infectious Diseases, Tokyo 162-8640, Japan. [28]Department of Veterinary Science, National Institute of Infectious Diseases, Tokyo 162-8640, Japan. [29]Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, MI 48109.

The spike (S) protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) plays a key role in viral infectivity. It is also the major antigen stimulating the host's protective immune response, specifically, the production of neutralizing antibodies. Recently, a new variant of SARS-CoV-2 possessing multiple mutations in the S protein, designated P.1, emerged in Brazil. Here, we characterized a P.1 variant isolated in Japan by using Syrian hamsters, a well-established small animal model for the study of SARS-CoV-2 disease (COVID-19). In hamsters, the variant showed replicative abilities and pathogenicity similar to those of early and contemporary strains (i.e., SARS-CoV-2 bearing aspartic acid [D] or glycine [G] at position 614 of the S protein). Sera and/or plasma from convalescent patients and BNT162b2 messenger RNA vaccinees showed comparable neutralization titers across the P.1 variant, S-614D, and S-614G strains. In contrast, the S-614D and S-614G strains were less well recognized than the P.1 variant by serum from a P.1-infected patient. Prior infection with S-614D or S-614G strains efficiently prevented the replication of the P.1 variant in the lower respiratory tract of hamsters upon reinfection. In addition, passive transfer of neutralizing antibodies to hamsters infected with the P.1 variant or the S-614G strain led to reduced virus replication in the lower respiratory tract. However, the effect was less pronounced against the P.1 variant than the S-614G strain. These findings suggest that the P.1 variant may be somewhat antigenically different from the early and contemporary strains of SARS-CoV-2.

6. Antibody-Dependent Enhancement of SARS-CoV-2 Infection Is Mediated by the IgG Receptors FcγRIIA and FcγRIIIA but Does Not Contribute to Aberrant Cytokine Production by Macrophages

Maemura T[1], Kuroda M[1], Armbrust T[1], Yamayoshi S, Halfmann PJ[1], Kawaoka Y.

The coronavirus disease 2019 (COVID-19) pandemic has raised concerns about the detrimental effects of antibodies. Antibody-dependent enhancement (ADE) of infection is one of the biggest concerns in terms of not only the antibody reaction to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) upon reinfection with the virus but also the reaction to COVID-19 vaccines. In this study, we evaluated ADE of infection by using COVID-19 convalescent-phase plasma and BHK cells expressing human Fcγ receptors (FcγRs). We found that FcγRIIA and FcγRIIIA mediated modest ADE of infection against SARS-CoV-2. Although ADE of infection was observed in monocyte-derived macrophages infected with SARS-CoV-2, including its variants, proinflammatory cytokine/chemokine expression was not upregulated in macrophages. SARS-CoV-2 infection thus produces antibodies that elicit ADE of infection, but these antibodies do not contribute to excess cytokine production by macrophages.

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Corporate Sponsored Research Program

Project Division of Fundamental Study on Cutting Edge of Genome Medicine

先端ゲノム医療の基盤研究寄付研究部門

Project Associate Professor　Hiroshi Yasui, M.D., D.M.Sc.　特任准教授　博士（医学）　安　井　　寛

Our major goal is to realize advanced genomic medicine. Major advancements in genome analysis have recently been reported from researchers around the globe, along with improvements in next-generation sequencing, leading to an era where genomic information can be collected and analyzed at low cost and in a short period of time. Thus, it is necessary to establish a framework for developing genome analysis while expanding our understanding of general society, academia, and medical associations, etc., in order to identify different diseases, such as hereditary diseases or non-genetic diseases.
In our department, comprehensive basic research on advanced genome medicine has been realized through our multidisciplinary collaborations among scientific experts; the Ethical, Legal, and Social Implications (ELSI) program; specimen preservation; electronic medical records access; and personal information protection.

1. Japan-US Comparative study for the promotion of the cancer genomic medicine in Japan

Hiroshi Yasui, Mikiko Suzuki, Arinobu Tojo[1]
[1]Tokyo Medical and Dental University

Regarding the spread of cancer genomic medicine Japan is behind not only Western countries but also China and Korea. We study to compare the current situation and the future prospects of cancer genomic medicine in Japan and the United States in order to contribute to design a policy to promote dissemination and uniformization of cancer genomic medicine for cancer patients in Japan.

2. Construction of infrastructure for research on advanced genome medicine

Hiroshi Yasui, Mikiko Suzuki, Arinobu Tojo[1]
[1]Tokyo Medical and Dental University

In order to establish a framework for developing genome analysis while expanding our understanding of diseases, including hereditary and nongenetic diseases, we are using comprehensive approaches to advanced genome medicine. These approaches include addressing various issues, such as multidisciplinary collaborations among scientific experts; the Ethical, Legal, and Social Implications (ELSI) program; management of specimen preservation, clinical information, and personal information protection for genomic medicine as well as biobanking. Our mission also includes enhancement of social acceptance for genomic medicine.

3. Program for supporting biospecimen analysis for the diagnosis and treatment of hematological malignancies

Hiroshi Yasui, Arinobu Tojo[1], Kaoru Uchimaru[2], Toshiki Watanabe[3]
[1]Tokyo Medical and Dental University

[2]Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo
[3]IMSUT hospital, The Institute of Medical Science, The University of Tokyo

To support cancer scientists in promoting translational research and genome medicine, we have established a platform for supporting cohort studies and biospecimen analysis. Under this program, we are collecting and managing clinical materials, including tumor cells, serum, and peripheral blood mononuclear cells from patients at high risk of hematologic malignancies as well as patients with blood cancer. We provide support for obtaining and/or analyzing biomaterials, as requested by researchers, and contribute to their clinical studies and publications.

4. Development of novel immunodiagnostics for graft-versus-host disease

Hiroshi Yasui, Asako Kobayashi, Reika Li, Takahiro Asatsuma, Mikiko Suzuki, Arinobu Tojo[1]
[1]Tokyo Medical and Dental University

Novel immunodiagnostics to analyze immune function is important for the evaluate the activity of autoimmune diseases as well as development of cancer immunotherapy. We study to develop novel immunodiagnostics to evaluate activities of immune cells in patients with allogenic hematopoietic stem cell transplantation to diagnose severity of graft-versus-host disease. It will be also expected to contribute the development of the novel cancer immunotherapy in hematologic malignancies.

5. Investigator-initiated clinical trials under an Investigational New Drug application for the development of novel cancer therapeutics and biomarkers

Hiroshi Yasui, Mikiko Suzuki, Kiyosumi Ochi, Fumitaka Nagamura[1], Giichiro Tsurita[2], Arinobu Tojo[3]:
[1]Center for Translational Research, IMSUT Hospital, The Institute of Medical Science, The University of Tokyo
[2]Department of Surgery, IMSUT Hospital, The Institute of Medical Science, The University of Tokyo
[3]Tokyo Medical and Dental University

Genome medicine and genome research, including pharmacogenomics and pharmacogenetics, are important for developing novel therapeutic agents for cancer and incurable diseases and identifying biomarkers. Our research aims to develop efficient approaches for conducting investigator-initiated clinical trials under Investigational New Drug (IND) applications to promote translational research and discover biomarkers for prediction of the safety and efficacy of novel therapeutics through omics analyses, including genomics. We were conducting, supporting, summarizing or preparing three investigator-initiated clinical trials under INDs applications for the development of academic-oriented innovative anticancer drug especially novel cancer immunotherapy.

6. Support and management of translational research

Hiroshi Yasui

To promote translational research and genome medicine, we participate in the "Translational Research Network Program, Japanese Translational Research and Clinical Trials Core Centers" supported by the Japan Agency for Medical Research and Development, as members of the Translational Research Advancement Center of the University of Tokyo. The aim of the program is to promote translational research and investigator-led clinical trials aiming for practical applications of basic studies in academia, managing the assessment of scientific seeds and intellectual property rights, and therefore promoting the development of advanced medical arts, including genome medicine.

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Social Cooperation Research Program

Project Division of RNA Medical Science
RNA 医科学社会連携研究部門

Project Associate Professor	Masaki Takahashi, Ph.D.		特任准教授	博士(理学)	高 橋 理 貴
Project Senior Assistant Professor	Kaku Goto, Ph.D.		特任講師	博士(理学)	後 藤 覚

RNA no longer stands behind DNA or protein but stands in front of DNA and protein. Recent achievements and discovery in biological science clearly emphasize the importance of RNA in life: the discovery of RNA interference, molecular mimicry between protein and RNA, ribosome structure at atomic resolution, and RNA quality control triggered by aberrant mRNAs. Moreover, the completed human genome project revealed, to our great surprise, the existence of a large amount of protein-noncoding RNAs (ncRNAs). These ncRNAs can be classified into two types: one, like antisense and microRNA, those function with sequence complementarity to the target mRNA or DNA, while the other, like aptamer, those function independent of sequence complementarity. In our laboratory, we aim to create artificial aptamers to target proteins of therapeutic interest.

The concept of using single-stranded nucleic acids (aptamers) as affinity molecules for protein or compound binding was initially described in 1990. The concept is based on the ability of short oligonucleotides to fold, in the presence of a target, into unique three-dimensional (3D) structures that bind the target with high affinity and specificity. Aptamers are generated by a process known as systematic evolution of ligands by exponential enrichment (SELEX), which merges combinatorial chemistry with in vitro evolution from a complex library of randomized $10^{14\text{-}15}$ different sequences. Importantly, aptamer targets can be small (e.g., chemical compounds) or large (e.g., proteins), and simple (e.g., purified proteins) or complex (e.g., protein complexes or cell surface receptors). Therefore, aptamers can be used as therapeutic compounds or reagents for affinity purification or as biosensor elements.

1. Nucleic acid ligands act as a PAM and agonist depending on the intrinsic ligand binding state of P2RY2.

Masaki Takahashi, Ryo Amano, Michiru Ozawa[1], Anna Martinez[1], Kazumasa Akita[1], Yoshikazu Nakamura[1]: [1]RIBOMIC Inc., Minato-ku, Tokyo

G-protein coupled receptors (GPCRs) play diverse roles in physiological processes, and hence the ligands to modulate GPCRs have served as worthwhile molecules in biological and pharmacological approaches. However, the exploration of novel ligands for GPCR still remains an arduous challenge. In this study, we report a method for the discovery of nucleic acid ligands against GPCRs by advanced RNA aptamer screening technology that employs a virus-like particle (VLP) exposing GPCR of interest and integrates high-throughput sequencing and bioinformatics. An array of biochemical analyses coupled with cell-based assay revealed that one of the aptamers raised against purinergic receptor P2Y2 (P2RY2), a

GPCR, exhibits an activation potency to unliganded receptor and prohibits a further receptor activation by endogenous ligand, behaving like a partial agonist. However, the aptamer enhances the activity of intrinsic ligand-binding P2RY2, thereby acting as a positive allosteric modulator (PAM) to liganded receptor. Our findings demonstrate that the nucleic acid aptamer conditionally exerts PAM and agonist effects on GPCRs depending on their intrinsic ligand binding state. These results indicate the validity of our VLP-based aptamer screening targeting GPCR and re-emphasize the high potential of nucleic acid ligands for exploring the GPCR activation mechanism and therapeutic applications.

2. An RNA aptamer restores defective bone growth in FGFR3-related skeletal dysplasia in mice

Takeshi Kimura[1], Michaela Bosakova[2,3,4], Yosuke Nonaka[5], Eva Hruba[4], Kie Yasuda[1], Satoshi Futakawa[5], Takuo Kubota[1], Bohumil Fafilek[2,3,4], Tomas Gregor[2,3], Sara P Abraham[2], Regina Gomolkova[2,4], Silvie Belaskova[3], Martin Pesl[2,3,6], Fabiana Csukasi[7,8], Ivan Duran[7,8], Masatoshi Fujiwara[5], Michaela Kavkova[9], Tomas Zikmund[9], Josef Kaiser[9], Marcela Buchtova[4,10], Deborah Krakow[7], Yoshikazu Nakamura[5], Keiichi Ozono[1], Pavel Krejci[2,3,4]:

[1]Department of Pediatrics, Osaka University Graduate School of Medicine, 565-0871 Osaka, Japan. [2]Department of Biology, Faculty of Medicine, Masaryk University, 62500 Brno, Czech Republic. [3]International Clinical Research Center, St. Anne's University Hospital, 65691 Brno, Czech Republic. [4]Institute of Animal Physiology and Genetics, Czech Academy of Sciences, 60200 Brno, Czech Republic. [5]RIBOMIC Inc., Tokyo 108-0071, Japan. [6]First Department of Internal Medicine-Cardioangiology, St. Anne's University Hospital, Masaryk University, 65691 Brno, Czech Republic. [7]Department of Orthopaedic Surgery, University of California Los Angeles, Los Angeles, CA 90095, USA. [8]Networking Research Center on Bioengineering, Biomaterials, and Nanomedicine (CIBER-BBN)-LABRET, University of Málaga, IBIMA-BIONAND, 29071 Málaga, Spain. [9]Central European Institute of Technology, Brno University of Technology, 61200 Brno, Czech Republic. [10]Department of Experimental Biology, Faculty of Science, Masaryk University, 62500 Brno, Czech Republic.

Achondroplasia is the most prevalent genetic form of dwarfism in humans and is caused by activating mutations in FGFR3 tyrosine kinase. The clinical need for a safe and effective inhibitor of FGFR3 is unmet, leaving achondroplasia currently incurable. Here, we evaluated RBM-007, an RNA aptamer previously developed to neutralize the FGFR3 ligand FGF2, for its activity against FGFR3. In cultured rat chondrocytes or mouse embryonal tibia organ culture, RBM-007 rescued the proliferation arrest, degradation of cartilaginous extracellular matrix, premature senescence, and impaired hypertrophic differentiation induced by FGFR3 signaling. In cartilage xenografts derived from induced pluripotent stem cells from individuals with achondroplasia, RBM-007 rescued impaired chondrocyte differentiation and maturation. When delivered by subcutaneous injection, RBM-007 restored defective skeletal growth in a mouse model of achondroplasia. We thus demonstrate a ligand-trap concept of targeting the cartilage FGFR3 and delineate a potential therapeutic approach for achondroplasia and other FGFR3-related skeletal dysplasias.

3. Multiple Therapeutic Applications of RBM-007, an Anti-FGF2 Aptamer.

Yoshikazu Nakamura[1]: [1]RIBOMIC Inc., Tokyo, Japan.

Vascular endothelial growth factor (VEGF) plays a pivotal role in angiogenesis, but is not the only player with an angiogenic function. Fibroblast growth factor-2 (FGF2), which was discovered before VEGF, is also an angiogenic growth factor. It has been shown that FGF2 plays positive pathophysiological roles in tissue remodeling, bone health, and regeneration, such as the repair of neuronal damage, skin wound healing, joint protection, and the control of hypertension. Targeting FGF2 as a therapeutic tool in disease treatment through clinically useful inhibitors has not been developed until recently. An isolated inhibitory RNA aptamer against FGF2, named RBM-007, has followed an extensive preclinical study, with two clinical trials in phase 2 and phase 1, respectively, underway to assess the therapeutic impact in age-related macular degeneration (wet AMD) and achondroplasia (ACH), respectively. Moreover, showing broad therapeutic potential, preclinical evidence supports the use of RBM-007 in the treatment of lung cancer and cancer pain.

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Social Cooperation Research Program

Project Division of International Advanced Medical Research

国際先端医療社会連携研究部門

Project Associate Professor　Koichiro Yuji, M.D., Ph.D.　特任准教授　博士（医学）　湯　地　晃一郎

The mission of the Project Division is to apply changes in advanced medical research at the Institute of Medical Science at the University of Tokyo (IMSUT). Our activities include field research in which innovative medicine will be implemented; cross-disciplinary education of physicians, researchers, and professionals; collaboration in innovative projects in the Coastal Area Life Innovation Comprehensive Special Zone for International Competitiveness Development; and establishing projections of the future healthcare system of Japan, which will be the first fully fledged aged society.

Implementing advanced medical research at IMSUT

Yuji, K.

The Project Division was established in November 2014. Our mission is to contribute to the progress of advanced medical research at IMSUT; to perform field research in which innovative medicine will be implemented; and to further the cross-disciplinary education of physicians, researchers, and professionals. Our future plans include collaboration in innovative projects in the Coastal Area Life Innovation Comprehensive Special Zone for International Competitiveness Development.

Projections on the future healthcare system in Japan, the first fully fledged aged society

Yuji, K.

Japan is rapidly becoming a fully fledged aged society, and the increasing dependence of the elderly population is a significant concern. We have simulated both the supply and demand features of Japan's future healthcare system.

Publications

1. Ikezawa K, Hirose M, Maruyama T, Yuji K, Yabe Y, Kanamori T, Kaide N, Tsuchiya Y, Hara S, Hideo S. Effect of early nutritional initiation on post-cerebral infarction discharge destination: A propensity-matched analysis using machine learning. Nutrition & Dietetics, in press.
2. 臨床遺伝専門医制度委員会　監修．臨床遺伝専門医テキストシリーズ分冊①　臨床遺伝学総論．（湯地晃一郎　分担執筆担当範囲：10．医療システムにおける遺伝医学：A 遺伝医療に関わる社会的規制，B 遺伝医療と医療経済，C 遺伝医療と医療安全）診断と治療社　2021年７月．

Social Cooperation Research Program

Project Division of Advanced Biopharmaceutical Science

先進的バイオ医薬品学社会連携研究部門

Professor	Kouhei Tsumoto, Ph.D.	教　授	博士(工学)	津　本　浩　平
Project Associate Professor	Satoru Nagatoishi, Ph.D.	特任准教授	博士(生命科学)	長門石　　曉

Various types of antibodies have been approved for therapeutic use and currently examined in clinical development. Therefore, developments of technology for the discovery and optimization of high-potency antibodies have been improved and have greatly increased to find the specific and stable antibody with desired biological properties. Biophysical analyses of a therapeutic antibody, particularly those of protein interaction and stability, are recognized as one of the critical procedures in the development of biopharmaceuticals, which would be assessed as an essential step to develop next-generation antibodies. The development of analytical methods with quantitative and high-sensitive detection of antigen interaction, protein stability, and biological function of antibody, therefore, has been intriguing for the pharmaceutic companies. In this division, we study biophysical analyses of various antibodies to propose a new strategy for the development of the next-generation antibody.

1. Proteomic identification and validation of novel interactions of the putative tumor suppressor PRELP with membrane proteins including IGFI-R and p75NTR

Kosuge H, Nakakido M, Nagatoishi S, Fukuda T, Bando Y, Ohnuma SI, Tsumoto K.

Proline and arginine-rich end leucine-rich repeat protein (PRELP) is a member of the small leucine-rich repeat proteoglycans (SLRPs) family. Levels of PRELP mRNA are downregulated in many types of cancer, and PRELP has been reported to have suppressive effects on tumor cell growth, although the molecular mechanism has yet to be fully elucidated. Given that other SLRPs regulate signaling pathways through interactions with various membrane proteins, we reasoned that PRELP likely interacts with membrane proteins to maintain cellular homeostasis. To identify membrane proteins that interact with PRELP, we carried out coimmunoprecipitation coupled with mass spectrometry (CoIP-MS). We prepared membrane fractions from Expi293 cells transfected to overexpress FLAG-tagged PRELP or control cells and analyzed samples precipitated with anti-FLAG antibody by mass spectrometry. Comparison of membrane proteins in each sample identified several that seem to interact with PRELP; among them, we noted two growth factor receptors, insulin-like growth factor I receptor (IGFI-R) and low-affinity nerve growth factor receptor (p75NTR), interactions with which might help to explain PRELP's links to cancer. We demonstrated that PRELP directly binds to extracellular domains of these two growth factor receptors with low micromolar affinities by surface plasmon resonance analysis using recombinant proteins. Furthermore, cell-based analysis using recombinant PRELP protein showed that PRELP suppressed cell growth and af-

fected cell morphology of A549 lung carcinoma cells, also at micromolar concentration. These results suggest that PRELP regulates cellular functions through interactions with IGFI-R and p75NTR and provide a broader set of candidate partners for further exploration

2. Heme controls the structural rearrangement of its sensor protein mediating the hemolytic bacterial survival

Nishinaga M, Sugimoto H, Nishitani Y, Nagai S, Nagatoishi S, Muraki N, Tosha T, Tsumoto K, Aono S, Shiro Y, Sawai H.

Hemes (iron-porphyrins) are critical for biological processes in all organisms. Hemolytic bacteria survive by acquiring b-type heme from hemoglobin in red blood cells from their animal hosts. These bacteria avoid the cytotoxicity of excess heme during hemolysis by expressing heme-responsive sensor proteins that act as transcriptional factors to regulate the heme efflux system in response to the cellular heme concentration. Here, the underlying regulatory mechanisms were investigated using crystallographic, spectroscopic, and biochemical studies to understand the structural basis of the heme-responsive sensor protein PefR from Streptococcus agalactiae, a causative agent of neonatal life-threatening infections. Structural comparison of heme-free PefR, its complex with a target DNA, and heme-bound PefR revealed that unique heme coordination controls a >20 Å structural rearrangement of the DNA binding domains to dissociate PefR from the target DNA. We also found heme-bound PefR stably binds exogenous ligands, including carbon monoxide, a by-product of the heme degradation reaction.

3. A DNA Aptamer That Inhibits the Aberrant Signaling of Fibroblast Growth Factor Receptor in Cancer Cells

Eguchi A, Ueki A, Hoshiyama J, Kuwata K, Chikaoka Y, Kawamura T, Nagatoishi S, Tsumoto K, Ueki R, Sando S.

Growth factor receptors are activated through dimerization by the binding of their ligands and play pivotal roles in normal cell function. However, the aberrant activity of the receptors has been associated with cancer malignancy. One of the main causes of the aberrant receptor activation is the overexpression of receptors and the resultant formation of unliganded receptor dimers, which can be activated in the absence of external ligand molecules. Thus, the unliganded receptor dimer is a promising target to inhibit aberrant signaling in cancer. Here, we report an aptamer that specifically binds to fibroblast growth factor receptor 2b and inhibits the aberrant receptor activation and signaling. Our investigation suggests that this aptamer inhibits the formation of the receptor dimer occurring in the absence of external ligand molecules. This work presents a new inhibitory function of aptamers and the possibility of oligonucleotide-based therapeutics for cancer.

4. Thermodynamic Dissection of Potency and Selectivity of Cytosolic Hsp90 Inhibitors.

Yoshimura C, Nagatoishi S, Kuroda D, Kodama Y, Uno T, Kitade M, Chong-Takata K, Oshiumi H, Muraoka H, Yamashita S, Kawai Y, Ohkubo S, Tsumoto K.

The cytosolic Hsp90-selective inhibitor TAS-116 has an acceptable safety profile and promising antitumor activity in clinical trials. We examined the binding characteristics of TAS-116 and its analogs to determine the impact of the ligand binding mode on selectivity for cytosolic Hsp90. Analyses of the co-crystal structure of Hsp90 and inhibitor TAS-116 suggest that TAS-116 interacts with the ATP-binding pocket, the ATP lid region, and the hydrophobic pocket. A competitive isothermal titration calorimetry analysis confirmed that a small fragment of TAS-116 (THS-510) docks into the lid region and hydrophobic pockets without binding to the ATP-binding pocket. THS-510 exhibited enthalpy-driven binding to Hsp90α and selectively inhibited cytosolic Hsp90 activity. The heat capacity change of THS-510 binding was positive, likely due to the induced conformational rearrangement of Hsp90. Thus, we concluded that interactions with the hydrophobic pocket of Hsp90 determine potency and selectivity of TAS-116 and derivatives for the cytosolic Hsp90 isoform

5. Elaboration of Non-naturally Occurring Helical Tripeptides as p53-MDM2/MDMX Interaction Inhibitors

Su A, Tabata Y, Aoki K, Sada A, Ohki R, Nagatoishi S, Tsumoto K, Wang S, Otani Y, Ohwada T.

Protein-protein interactions (PPIs) are often mediated by helical, strand and/or coil secondary structures at the interface regions. We previously showed that non-naturally occurring, stable helical trimers of bicyclic β-amino acids (Abh) with all-trans amide bonds can block the p53-MDM2/MDMX α-helix-helix interaction, which plays a role in regulating p53 function. Here, we conducted docking and molecular dynamics calculations to guide the structural optimization of our reported compounds, focusing on modifications of the C-terminal/N-terminal residues. We confirmed that the modified peptides directly bind to MDM2 by means of thermal shift assay, isothermal titration calorimetry, and enzyme-linked immunosorbent assay (ELISA) experiments. Biological

activity assay in human osteosarcoma cell line SJSA-1, which has wild-type p53 and amplification of the Mdm2 gene, indicated that these peptides are membrane-permeable p53-MDM2/MDMX interaction antagonists that can rescue p53 function in the cells.

6. Anion solvation enhanced by positive supercharging mutations preserves thermal stability of an antibody in a wide pH range

Kasahara K, Kuroda D, Tanabe A, Kawade R, Nagatoishi S, Tsumoto K.

Proteins function through interactions with other molecules. In protein engineering, scientists often engineer proteins by mutating their amino acid sequences on the protein surface to improve various physicochemical properties. "Supercharging" is a method to design proteins by mutating surface residues with charged amino acids. Previous studies demonstrated that supercharging mutations conferred better thermal resistance, solubility, and cell penetration to proteins. Likewise, antibodies recognize antigens through the antigen-binding site on the surface. The genetic and structural diversity of antibodies leads to high specificity and affinity toward antigens, enabling antibodies to be versatile tools in various applications. When assessing therapeutic antibodies, surface charge is an important factor to consider because the isoelectric point plays a role in protein clearance inside the body. In this study, we explored how supercharging mutations affect physicochemical properties of antibodies. Starting from a crystal structure of an antibody with the net charge of -4, we computationally designed a supercharged variant possessing the net charge of +10. The positive-supercharged antibody exhibited marginal improvement in thermal stability, but the secondary structure and the binding affinity to the antigen (net charge of +8) were preserved. We also used physicochemical measurements and molecular dynamics simulations to analyze the effects of supercharging mutations in sodium phosphate buffer with different pH and ion concentrations, which revealed preferential solvation of phosphate ions to the supercharged surface relative to the wild-type surface. These results suggest that supercharging would be a useful approach to preserving thermal stability of antibodies in a wide range of pH, which may enable further diversification of antibody repertoires beyond natural evolution.

7. A glutamine sensor that directly activates TORC1

Tanigawa M, Yamamoto K, Nagatoishi S, Nagata K, Noshiro D, Noda NN, Tsumoto K, Maeda T.

TOR complex 1 (TORC1) is an evolutionarily-conserved protein kinase that controls cell growth and metabolism in response to nutrients, particularly amino acids. In mammals, several amino acid sensors have been identified that converge on the multi-layered machinery regulating Rag GTPases to trigger TORC1 activation; however, these sensors are not conserved in many other organisms including yeast. Previously, we reported that glutamine activates yeast TORC1 via a Gtr (Rag ortholog)-independent mechanism involving the vacuolar protein Pib2, although the identity of the supposed glutamine sensor and the exact TORC1 activation mechanism remain unclear. In this study, we successfully reconstituted glutamine-responsive TORC1 activation in vitro using only purified Pib2 and TORC1. In addition, we found that glutamine specifically induced a change in the folding state of Pib2. These findings indicate that Pib2 is a glutamine sensor that directly activates TORC1, providing a new model for the metabolic control of cells.

8. Mechanism of dimerization and structural features of human LI-cadherin

Yui A, Caaveiro JMM, Kuroda D, Nakakido M, Nagatoishi S, Goda S, Maruno T, Uchiyama S, Tsumoto K

Liver intestine (LI)-cadherin is a member of the cadherin superfamily, which encompasses a group of Ca2 + -dependent cell-adhesion proteins. The expression of LI-cadherin is observed on various types of cells in the human body, such as normal small intestine and colon cells, and gastric cancer cells. Because its expression is not observed on normal gastric cells, LI-cadherin is a promising target for gastric cancer imaging. However, because the cell adhesion mechanism of LI-cadherin has remained unknown, rational design of therapeutic molecules targeting this cadherin has been hampered. Here, we have studied the homodimerization mechanism of LI-cadherin. We report the crystal structure of the LI-cadherin homodimer containing its first four extracellular cadherin repeats (EC1-4). The EC1-4 homodimer exhibited a unique architecture different from that of other cadherins reported so far, driven by the interactions between EC2 of one protein chain and EC4 of the second protein chain. The crystal structure also revealed that LI-cadherin possesses a noncanonical calcium ion-free linker between the EC2 and EC3 domains. Various biochemical techniques and molecular dynamics simulations were employed to elucidate the mechanism of homodimerization. We also showed that the formation of the homodimer observed in the crystal structure is necessary for LI-cadherin-dependent cell adhesion by performing cell aggregation assays. Taken together, our data provide structural insights necessary to advance the use of LI-cadherin as a target for imaging gastric cancer.

9. Regulation of cadherin dimerization by chemical fragments as a trigger to inhibit cell adhesion

Senoo A, Ito S, Nagatoishi S, Saito Y, Ueno G, Kuroda D, Yoshida K, Tashima T, Kudo S, Sando S, Tsumoto K.

Many cadherin family proteins are associated with diseases such as cancer. Since cell adhesion requires homodimerization of cadherin molecules, a small-molecule regulator of dimerization would have therapeutic potential. Herein, we describe identification of a P-cadherin-specific chemical fragment that inhibits P-cadherin-mediated cell adhesion. Although the identified molecule is a fragment compound, it binds to a cavity of P-cadherin that has not previously been targeted, indirectly prevents formation of hydrogen bonds necessary for formation of an intermediate called the X dimer and thus modulates the process of X dimerization. Our findings will impact on a strategy for regulation of protein-protein interactions and stepwise assembly of protein complexes using small molecules.

10. Development of biparatopic bispecific antibody possessing tetravalent scFv-Fc capable of binding to ROBO1 expressed in hepatocellular carcinoma cells

Watanabe Y, Tanabe A, Hamakubo T, Nagatoishi S, Tsumoto K.

There is no standard structural format of the biparatopic bispecific antibody (bbsAb) which is used against the target molecule because of the diversity of biophysical features of bispecific antibodies (bsAbs). It is therefore essential that the interaction between the antibody and antigen is quantitatively analyzed to design antibodies that possess the desired properties. Here, we generated bsAbs, namely, a tandem scFv-Fc, a diabody-Fc, and an immunofusion-scFv-Fc-scFv, that possessed four scFv arms at different positions and were capable of recognizing the extracellular domains of ROBO1. We examined the interactions between these bsAbs and ROBO1 at the biophysical and cellular levels. Of these, immunofusion-B2212A scFv-Fc-B5209B scFv was stably expressed with the highest relative yield. The kinetic and thermodynamic features of the interactions of each bsAb with soluble ROBO1 (sROBO1) were validated using surface plasmon resonance and isothermal titration calorimetry. In all bsAbs, the immunofusion-scFv-Fc-scFv format showed homogeneous interaction with the antigen with higher affinity compared with that of monospecific antibodies. In conclusion, our study presents constructive information to design druggable bbsAbs in drug applications.

11. Electrostatic-triggered exothermic antibody adsorption to the cellulose nanoparticles

Murakami K, Nagatoishi S, Kasahara K, Nagai H, Sasajima Y, Sasaki R, Tsumoto K.

Antibody-conjugated nanoparticles are used in a fields ranging from medicine to engineering. NanoAct® nanobeads are cellulose nanoparticles used in lateral flow assays that are highly water dispersible. In order to promote the adsorption of antibodies onto NanoAct® particles while maintaining their activity, we analyzed the adsorption onto NanoAct® particles thermodynamically and elucidated the adsorption mechanism. In an immunochromatographic assay, the amount of adsorbed antibody and the color intensity of the test line increased as the pH decreased. The zeta potential of the nanoparticles remained constant at around -30 mV over the pH range from 2 to 10. The model antibody had pI values between 6.2 and 6.8. Isothermal calorimetry analysis showed that adsorption of antibody to the NanoAct® particle is an endothermic reaction under low pH conditions, an exothermic reaction between pH 6 and pH 7, and a weakly exothermic reaction above pH 7. These data indicate that the changes in net charge of the antibody surface as a function of pH influence the pH dependence of antibody adsorption to the negatively charged NanoAct®. This suggests that increased positive charge on the antibody surface will result in a more sensitive NanoAct®-based immunoassay.

12. An integrated computational pipeline for designing high-affinity nanobodies with expanded genetic codes

Padhi AK, Kumar A, Haruna KI, Sato H, Tamura H, Nagatoishi S, Tsumoto K, Yamaguchi A, Iraha F, Takahashi M, Sakamoto K, Zhang KYJ.

Protein engineering and design principles employing the 20 standard amino acids have been extensively used to achieve stable protein scaffolds and deliver their specific activities. Although this confers some advantages, it often restricts the sequence, chemical space, and ultimately the functional diversity of proteins. Moreover, although site-specific incorporation of non-natural amino acids (nnAAs) has been proven to be a valuable strategy in protein engineering and therapeutics development, its utility in the affinity-maturation of nanobodies is not fully explored. Besides, current experimental methods do not routinely employ nnAAs due to their enormous library size and infinite combinations. To address this, we have developed an integrated computational pipeline employing structure-based protein design methodologies, molecular dynamics simulations and free energy calculations, for the binding affinity prediction of an nnAA-incorporated nanobody toward

its target and selection of potent binders. We show that by incorporating halogenated tyrosines, the affinity of 9G8 nanobody can be improved toward epidermal growth factor receptor (EGFR), a crucial cancer target. Surface plasmon resonance (SPR) assays showed that the binding of several 3-chloro-l-tyrosine (3MY)-incorporated nanobodies were improved up to 6-fold into a picomolar range, and the computationally estimated binding affinities shared a Pearson's r of 0.87 with SPR results. The improved affinity was found to be due to enhanced van der Waals interactions of key 3MY-proximate nanobody residues with EGFR, and an overall increase in the nanobody's structural stability. In conclusion, we show that our method can facilitate screening large libraries and predict potent site-specific nnAA-incorporated nanobody binders against crucial disease-targets.

13. The transcriptional corepressor CtBP2 serves as a metabolite sensor orchestrating hepatic glucose and lipid homeostasis

Ota T, Senoo A, Shirakawa M, Nonaka H, Saito Y, Ito S, Ueno G, Nagatoishi S, Tsumoto K, Sando S.

Biological systems to sense and respond to metabolic perturbations are critical for the maintenance of cellular homeostasis. Here we describe a hepatic system in this context orchestrated by the transcriptional corepressor C-terminal binding protein 2 (CtBP2) that harbors metabolite-sensing capabilities. The repressor activity of CtBP2 is reciprocally regulated by NADH and acyl-CoAs. CtBP2 represses Forkhead box O1 (FoxO1)-mediated hepatic gluconeogenesis directly as well as Sterol Regulatory Element-Binding Protein 1 (SREBP1)-mediated lipogenesis indirectly. The activity of CtBP2 is markedly defective in obese liver reflecting the metabolic perturbations. Thus, liver-specific CtBP2 deletion promotes hepatic gluconeogenesis and accelerates the progression of steatohepatitis. Conversely, activation of CtBP2 ameliorates diabetes and hepatic steatosis in obesity. The structure-function relationships revealed in this study identify a critical structural domain called Rossmann fold, a metabolite-sensing pocket, that is susceptible to metabolic liabilities and potentially targetable for developing therapeutic approaches

14. Structural and thermodynamical insights into the binding and inhibition of FIH-1 by the N-terminal disordered region of Mint3

Ten T, Nagatoishi S, Maeda R, Hoshino M, Nakayama Y, Seiki M, Sakamoto T, Tsumoto K.

Mint3 is known to enhance aerobic ATP production, known as the Warburg effect, by binding to FIH-1. Since this effect is considered to be beneficial for cancer cells, the interaction is a promising target for cancer therapy. However, previous research has suggested that the interacting region of Mint3 with FIH-1 is intrinsically disordered, which makes investigation of this interaction challenging. Therefore, we adopted thermodynamic and structural studies in solution to clarify the structural and thermodynamical changes of Mint3 binding to FIH-1. First, using a combination of circular dichroism, nuclear magnetic resonance, and hydrogen/deuterium exchange-mass spectrometry (HDX-MS), we confirmed that the N-terminal half, which is the interacting part of Mint3, is mostly disordered. Next, we revealed a large enthalpy and entropy change in the interaction of Mint3 using isothermal titration calorimetry (ITC). The profile is consistent with the model that the flexibility of disordered Mint3 is drastically reduced upon binding to FIH-1. Moreover, we performed a series of ITC experiments with several types of truncated Mint3s, an effective approach since the interacting part of Mint3 is disordered, and identified amino acids 78 to 88 as a novel core site for binding to FIH-1. The truncation study of Mint3 also revealed the thermodynamic contribution of each part of Mint3 to the interaction with FIH-1, where the core sites contribute to the affinity (ΔG), while other sites only affect enthalpy (ΔH), by forming noncovalent bonds. This insight can serve as a foothold for further investigation of intrinsically disordered regions (IDRs) and drug development for cancer therapy.

15. Structure-based screening combined with computational and biochemical analyses identified the inhibitor targeting the binding of DNA Ligase 1 to UHRF1

Kori S, Shibahashi Y, Ekimoto T, Nishiyama A, Yoshimi S, Yamaguchi K, Nagatoishi S, Ohta M, Tsumoto K, Nakanishi M, Defossez PA, Ikeguchi M, Arita K.

The accumulation of epigenetic alterations is one of the major causes of tumorigenesis. Aberrant DNA methylation patterns cause genome instability and silencing of tumor suppressor genes in various types of tumors. Therefore, drugs that target DNA methylation-regulating factors have great potential for cancer therapy. Ubiquitin-like containing PHD and RING finger domain 1 (UHRF1) is an essential factor for DNA methylation maintenance. UHRF1 is overexpressed in various cancer cells and down-regulation of UHRF1 in these cells reactivates the expression of tumor suppressor genes, thus UHRF1 is a promising target for cancer therapy. We have previously shown that interaction between the tandem Tudor domain (TTD) of UHRF1 and DNA ligase 1 (LIG1) di/trimethylated on Lys126 plays a key role in the recruitment of UHRF1 to replication sites and replication-coupled DNA methylation maintenance. An arginine binding cavity (Arg-binding cavity) of the TTD is essential for

LIG1 interaction, thus the development of inhibitors that target the Arg-binding cavity could potentially repress UHRF1 function in cancer cells. To develop such an inhibitor, we performed in silico screening using not only static but also dynamic metrics based on all-atom molecular dynamics simulations, resulting in efficient identification of 5-amino-2,4-dimethylpyridine (5A-DMP) as a novel TTD-binding compound. Crystal structure of the TTD in complex with 5A-DMP revealed that the compound stably bound to the Arg-binding cavity of the TTD. Furthermore, 5A-DMP inhibits the full-length UHRF1:LIG1 interaction in Xenopus egg extracts. Our study uncovers a UHRF1 inhibitor which can be the basis of future experiments for cancer therapy.

Publications

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Social Cooperation Research Program

Project Division of Cancer Biomolecular Therapy
がん生体分子治療社会連携研究部門

Project Professor	Hideaki Tahara, M.D., Ph.D.		特任教授	医学博士	田原　秀晃
Project Associate Professor	Hiroaki Uchida, M.D., Ph.D.		特任准教授	博士（医学）	内田　宏昭

Our division has been conducting basic research projects for development of innovative cancer therapy using immunologic and gene therapy approaches. The reagents, modalities, and concepts developed in this division have been clinically applied as translational research projects. We believe that bidirectional information exchange between the bench and the bedside would be one of the most important requirements for the successful development of novel and effective therapies.

I. Development of cancer immunotherapy using the blockade of MFG-E8

Mika Uematsu-Hamada, Yu Mizote[1], Miho Kudo, Hiroaki Uchida, Hideaki Tahara ([1]Department of Cancer Drug Discovery and Development, Osaka International Cancer Institute)

The secreted protein, milk fat globule-EGF factor 8 (MFG-E8), stimulates disease progression through coordinated αvβ3 integrin signaling in tumor and host cells. MFG-E8 enhances tumor cell survival, invasion, and angiogenesis, and contributes to local immune suppression.

We have shown that systemic MFG-E8 blockade cooperates with cytotoxic chemotherapy, molecularly targeted therapy, and radiation therapy to induce destruction of various types of established mouse tumors. The combination treatments evoke extensive tumor cell apoptosis that is coupled to efficient dendritic cell cross-presentation of dying tumor cells. Our previous findings suggest that systemic MFG-E8 blockade might intensify the antitumor activities of existing therapeutic regimens through coordinated cell-autonomous. Our subsequent studies on human samples suggest that MFG-E8 derived from the tumor cells might affect the outcome of the chemotherapy for cancer patients. Based on these findings, our recent projects include the investigation on the significance of tumor-derived and non-tumor derived MFG-E8 using MFG-E8 gene knock-out mice. Furthermore, we are now seeking the mechanisms for the upregulation of immune-regulatory molecules including PD-L1 and MFG-E8 using human cancer cell lines. We believe that these efforts will results in the development of new class of anti-tumor therapies.

II. Development of fully retargeted herpes simplex virus (HSV) vectors for oncolytic virotherapy

Hiroaki Uchida, Hitomi Ikeda, Tomoko Shibata, Takuma Suzuki, Fumihiro Nagata, Natsuki Matsumoto, Yukinari Kato[2], Hideaki Tahara ([2]Department of Antibody Drug Development, Tohoku University Graduate School of Medicine)

Herpes simplex virus (HSV) vectors are promising agents for oncolytic virotherapy. Uchida established a fully retargeted HSV platform that mediates virus entry exclusively via tumor-associated antigens in the lab of Prof. Joseph Glorioso at the University of Pittsburgh. Entry of HSV is initiated by the binding of glycoprotein D (gD) to one of its receptors, herpesvirus entry mediator (HVEM) or nectin-1. This interaction results in a conformational change in gD, triggering

sequential activation of gH and gB to execute fusion between the viral envelope and cell membranes. We inserted single-chain antibodies (scFv) against a number of different cell surface molecules such as epidermal growth factor receptor (EGFR), carcinoembryonic antigen (CEA), and epithelial cell adhesion molecule (EpCAM), into the retargeted HSV platform that encodes a gD ablated for binding to natural receptors and a gB containing entry-enhancing mutations we previously identified. As a result, we observed specific virus entry into cells expressing the cognate target antigen for each of the retargeted constructs. Our results indicate the adaptability of our system to different targeting ligands, leading to a new generation of broadly applicable and effective oncolytic HSV vectors (receptor-retargeted oncolytic HSVs; RR-oHSVs). Furthermore, we introduced syncytial mutations into the gB and/or gK genes of RR-oHSVs and found that gD retargeting does not abolish the hyperfusogenic activity of syncytial mutations and that these mutations do not eliminate the dependence of HSV entry and spread on a specific gD-receptor interaction. We investigated the *in vivo* anti-tumor effects of the RR-oHSVs that harbor the syncytial mutations (RRsyn-oHSVs) using human cancer xenografts in immunodeficient mice. With only a single intra-tumoral injection of RRsyn-oHSV at very low doses, all treated tumors regressed completely. Furthermore, intra-venous administration of RRsyn-oHSV resulted in robust anti-tumor effects even against large tumors. We found that these potent anti-tumor effects of RRsyn-oHSV may be associated with the formation of long-lasting tumor cell syncytia not containing non-cancerous cells that appear to trigger death of the syncytia. These results strongly suggest that cancer patients with distant metastases could be effectively treated with our RRsyn-oHSV. Additionally, we are developing novel oncolytic vectors that are retargeted to tumor-associated antigens that have been shown to be expressed specifically on cancer cells.

III. Establishment of highly functional monoclonal antibodies through novel screening methods for targeted cancer therapy

Hiroaki Uchida, Hitomi Ikeda, Tomoko Shibata, Miki Yamaguchi[3], Hideaki Tahara ([3]Department of Molecular Medicine, Research Institute for Frontier Medicine, Sapporo Medical University School of Medicine)

Monoclonal antibodies (mAbs) have become an established therapeutic modality in clinical oncology. In order to identify cell-surface molecules that may be useful for targeting various types of cancers, our group established a unique screening approach that employs an adenoviral vector harboring fiber proteins engineered to bind antibodies, Adv-FZ33. This approach led to the successful identification of an array of potential target molecules for cancer treatment. Immunotoxins (antibody-drug conjugates; ADC) are a promising class of cancer therapeutics composed of a cytotoxic agent linked covalently to a cancer-targeted antibody. To systematically hunt for cell-surface molecules that may be efficiently targeted by immunotoxins, our group created another method for screening highly functional cancer-targeted mAbs and cognate antigens. The receptor-binding domain of the Diphtheria toxin (DT) was replaced with the antibody-binding domain (3C) derived from the Streptococcal protein G. The resultant mutated toxin protein (DT-3C) was used for selection of mAbs for specific cell killing activity as components of immunotoxins. Our novel screening system is advantageous in that the selected antibodies bind to intact cancer cells and get internalized efficiently, which has been critically required for therapeutic applications but elusive thus far. Furthermore, we have successfully taken advantage of some of these in-house monoclonal antibodies for development of novel fully retargeted HSV vectors. Additionally, we have created an HSV-based probe for screening of Abs that could mediate HSV entry by recognition of unknown receptors. We have found that one of the Abs selected by this screening method is capable of mediating HSV entry when incorporated into gD as an scFv. Interestingly, the antigen recognized by the Ab has been found to be epiregulin, a molecule that is known as a growth factor expressed and shed from cancer cells. This was unexpected because this molecule has been commonly investigated as a soluble "ligand" that acts as a growth factor, and thus not as a membrane-bound "receptor". Thus, we expect that this novel Ab-screening system may lead to a new generation of RR-oHSV vectors.

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Social Cooperation Research Program

Project Division of Genomic Medicine and Disease Prevention

ゲノム予防医学社会連携研究部門

Project Professor	Toru Suzuki, M.D., Ph.D.		特任教授	博士(医学)	鈴木　亨
Professor	Yoshinori Murakami, M.D., Ph.D.		教　授	医学博士	村上善則

Diseases, including cancer and common/chronic conditions, develop/progress by the combination/interaction of genetic background, acquired environmental exposures, life-style factors and aging. Identification of risk factors at time of birth and later in life provides information on which approaches to disease prevention can be tailored. The Project Division of Genomic Medicine and Disease Prevention was started on July 1, 2019 in cooperation with Nippon Telegram and Telephone Cooperation (NTT), with a goal to develop personalized/precision-based prevention of diseases by integrating genomic information, health records and life-style data.

1. Towards the development of personalized and precision-based prevention of diseases

Toru Suzuki[1], Takayuki Morisaki[1], Atsuko Hiraishi[1], Masaru Koido[2], Momoko Horikoshi[3], Yoichiro Kamatani[2], Yoshinori Murakami[1]; [1]Project Division of Genomic Medicine and Disease Prevention, The Institute of Medical Science, the University of Tokyo, [2]Laboratory of Complex Trait Genomics, Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, the University of Tokyo, Tokyo. [3]Laboratory for Endocrinology, Metabolism and Kidney Diseases, RIKEN Centre for Integrative Medical Sciences,

The Project Division of Genetic Medicine and Disease Prevention was established in 2019 to obtain scientific evidence to enable disease prevention by integrating genetic information into healthcare-based information (eg health records), life-style data and age.

For this purpose, a collaborative project with NTT Life Science, Corp. was initiated in 2020 to undertake research to integrate genetic testing with healthcare data to identify disease risk. Employees of NTT group who undertake regular/annual physical examinations were recruited to a comprehensive survey program of genetic testing using microarray analysis. The program aims to investigate use of polygenic risk scores to identify genetic risk of conditions, and to share information with participants to potentially improve/intervene through lifestyle modifications improvement in a manner that is compliant in terms of ethical, legal and social issues (ELSI). This project is being undertaken in collaboration with several hospitals, including the Center for Disease Prevention at the NTT Medical Center, Tokyo. Integration of genetic information into health records and re-evaluation of disease risks of individuals are also being examined.

In 2021, a grant from the Japanese Science and Technology Agency's JST-Mirai Program on Advanced Intelligent Information Society mission area (Human centric digital twins services) was awarded to investigate "Development of disease prevention systems by integrating multi-layered biomedical information". The prioritized theme targets individuals and organizations as components of society, using the

premise of AI digital twin as its core, and aims to: (1) create new value for emerging needs and issues, and (2) propose and realize new concepts and services related to AI digital twin. Specifically, it aims at an optimal combination of technologies related to data collection, processing, conversion, and integration, which are the prerequisites for digital twinning, as well as data conversion technologies suitable for modern AI technology, intelligent integration of output results, etc., with an eye toward the future of services, in addition to the advancement of individual core technologies.

The present study aims to enable inclusion of multi-layered bio-medical information into current company-based health care systems to develop a next-generation health care system, and reflects the core mission of the Division.

Workstreams include -

1. Longitudinal health care information of employees in a company-based cohort in Japan;

2. SNP typing information of individuals (with informed consent) to generate polygenic risk scores of various diseases; and

3. Multiomics information, such as information on the metabolome.

This project aims to integrate these multi-layered bio-medical information into a digital twin model of individuals in a middle- aged/working generation, and to develop novel algorithms to predict risk of various polygenic diseases in the Japanese population.

The Division aims to conduct a symposium to review interim results in June 2022.

Representative publications -

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Social Cooperation Research Program

Project Division of Clinical Precision Research Platform

臨床精密研究基盤社会連携研究部門

Project Professor　Satoshi Takahashi, M.D., D.M.Sc.　特任教授　博士（医学）　高　橋　　聡

We have opened a new lab in April 2021 under a joint research agreement with Daiichi Sankyo RD Novare Co., Ltd. Our research objectives are to conduct precision research for the development of precision medicine, to combine comprehensive multi-omics analysis including clinical epigenomics and single-cell sequencing with drug sensitivity testing in hematopoietic diseases and colorectal cancer, to study each method for these integrated data analysis, and to understand diseases and create new treatments. Missions for this include conducting multi-layered and comprehensive clinical sequencing, optimizing methods for processing small amounts of clinical specimens, promoting miniaturization and automation of drug sensitivity test, and model establishment, and conducting research that leads to clinical applications.

1. Clinical precision research for hematological diseases by genomic and multi omics analysis

Hironobu Komori[1,2], Hayato Tsuji[1,2], Yoshiharu Takama[1,2], Seiko Kato[1], Maiko Morita[1], Sanae Suzuki[1], Tetsushi Oka[2], Yoshimasa Ono[2], Yasuo Nagasawa[2], Kenji Wakabayashi[2], Gen Kudo[2], Satoshi Takahashi[1].

[1] IMSUT, [2] Daiichi Sankyo RD Novare Co., Ltd.

In this study, we will conduct a comprehensive multi-omics analysis of genetic mutations, epigenetic changes, and protein expression and modification of blood cells from individual patients with hematopoietic malignancies or other hematopoietic diseases who visited the IMSUT hospital or collaborating hospitals, together with the Department of Hematology and Oncology and other collaborators. In addition, we aim to develop more precise medicine by conducting drug sensitivity tests for chemotherapy using cells derived from patients with hematopoietic malignancies. For this purpose, we are conducting whole genome analysis of hematopoietic malignant cells, RNA expression analysis, transcriptome analysis, DNA methylation analysis, genome structure analysis, etc., aiming to analyze these at the single cell level, immunostaining, western blot analysis, and others. In addition, sensitivity tests to anti-tumor drugs, including new anti-cancer drug candidates, will be conducted. We are planning to analyze this information in an integrated manner to promote more precise pathophysiological analysis, to search for factors related to drug sensitivity, and to construct an analysis system that can return the analysis results to clinic to assist in the determination of treatment strategies in real time. In addition, we would plan to analyze immune cells surrounding hematopoietic tumor cells to obtain knowledge useful for new treatment and prevention.

This research is expected to contribute to the discovery of biomarkers that indicate the characteristics of hematopoietic insufficiency syndrome and hematopoietic malignancies, the development of preci-

sion medicine tailored to patients' diseases, and the development of new therapeutics and preventive drugs/ methodologies.

2. Generation of antigen-specific T cells derived from cord blood

Morita Maiko[1], Seiko Kato[1], Satoshi Yamazaki[1,2], Ai Tachikawa-Kawana[1,3], Patrick Hanley[4], Catherin Bollard[4], Satoshi Takahashi[1]

[1] IMSUT, [2] University of Tsukuba, [3] National Institute of Infectious Diseases, [4] Children's National Research Institute

The aim of this project is to establish a method for generating and amplifying antigen-specific T cells from cord blood-derived naive T cells using methods other than gene transfer.

We are working to optimize an effective priming method for cord blood-derived antigen-presenting cells using pattern recognition receptors, such as STING ligands or TLR8, and to establish a method for generating and amplifying antigen-specific T cells from immunologically naive T cells. The goal is to advance this research and plan future clinical studies to improve the safety of cord blood transplantation by promoting immune reconstitution against viral infections after transplantation.

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Social Cooperation Research Program

Project Division of Innovative Diagnostics Technology Platform

革新的診断技術応用基盤社会連携研究部門

Project Associate Professor　Hiroshi Yasui, M.D., D.M.Sc.　特任准教授　博士（医学）　安　井　　寛

In this laboratory, we aim to create innovative diagnostic technologies by combining various analysis and measurement technologies with ideas and unmet needs from the clinical perspective as hematologists-oncologists. We will provide innovative approaches to intractable diseases and conditions, and study the optimization of diagnosis and treatment.

1. Research and development of novel diagnostics to evaluate immune response

Hiroshi Yasui, Muneyoshi Futami, Asako Kobayashi, Reika Li, Mikiko Suzuki, Keiji Hirano[1], Yuma Oka[1], Masatoshi Yanagida[1], Arinobu Tojo[2]
[1]Central Research Laboratories, Sysmex Corporation
[2]Tokyo Medical and Dental University

Novel immunodiagnostics to analyze immune function is important for the evaluate the activity of autoimmune diseases as well as development of cancer immunotherapy. We study to develop novel immunodiagnostics to evaluate activities of immune cells in patients with allogenic hematopoietic stem cell transplantation to diagnose severity of graft-versus-host disease. It will be also expected to contribute the development of the novel cancer immunotherapy in hematologic malignancies.

2. Investigator-initiated clinical trials under an Investigational New Drug application for the development of novel cancer therapeutics and biomarkers

Hiroshi Yasui, Mikiko Suzuki, Kiyosumi Ochi, Fumitaka Nagamura[1], Giichiro Tsurita[2], Arinobu Tojo[3]:
[1]Center for Translational Research, IMSUT Hospital, The Institute of Medical Science, The University of Tokyo
[2]Department of Surgery, IMSUT Hospital, The Institute of Medical Science, The University of Tokyo
[3]Tokyo Medical and Dental University

Genome medicine and genome research, including pharmacogenomics and pharmacogenetics, are important for developing novel therapeutic agents for cancer and incurable diseases and identifying biomarkers. Our research aims to develop efficient approaches for conducting investigator-initiated clinical trials under Investigational New Drug (IND) applications to promote translational research and discover biomarkers for prediction of the safety and efficacy of novel therapeutics through omics analyses, including genomics. We were conducting, supporting, summarizing or preparing three investigator-initiated clinical trials under INDs applications for the development of academic-oriented innovative anticancer drug especially novel cancer immunotherapy.

3. Program for supporting biospecimen analysis for the diagnosis and treatment of hematological malignancies

Hiroshi Yasui, Arinobu Tojo[1], Kaoru Uchimaru[2], Toshiki Watanabe[3]
[1]Tokyo Medical and Dental University
[2]Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo
[3]IMSUT hospital, The Institute of Medical Science, The University of Tokyo

To support cancer scientists in promoting translational research and genome medicine, we have established a platform for supporting cohort studies and biospecimen analysis. Under this program, we are collecting and managing clinical materials, including tumor cells, serum, and peripheral blood mononuclear cells from patients at high risk of hematologic malignancies as well as patients with blood cancer. We provide support for obtaining and/or analyzing biomaterials, as requested by researchers, and contribute to their clinical studies and publications.

4. Support and management of translational research

Hiroshi Yasui

To promote translational research and genome medicine, we participate in the "Translational Research Network Program, Japanese Translational Research and Clinical Trials Core Centers" supported by the Japan Agency for Medical Research and Development, as members of the Translational Research Advancement Center of the University of Tokyo. The aim of the program is to promote translational research and investigator-led clinical trials aiming for practical applications of basic studies in academia, managing the assessment of scientific seeds and intellectual property rights, and therefore promoting the development of advanced medical arts, including genome medicine.

Publications

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2. Osada N, Kikuchi J, Koyama D, Kuroda Y, Yasui H, Leverson JD, Furukawa Y. mTOR inhibitors sensitize multiple myeloma cells to venetoclax via IKZF3- and Blimp-1-mediated BCL-2 up-regulation *Haematologica*, 2021, Nov 1;106(11):3008-3013. doi: 10.3324/haematol.2021.278506.
3. Yamaguchi R, Yasui H. A Future Perspective on Cancer Precision Medicine Accelerated by AI and Data Science *Gan To Kagaku Ryoho.* 2021 Dec; 48(12): 1415-1419.

Dean's Office

Project Coordination Office
プロジェクトコーディネーター室

Professor Makoto Nakanishi, M.D., Ph.D.

教 授 医学博士 中 西 真

Our major missions are to coordinate institutional projects, enhance the mutual cooperation, alliance among teaching and research staff, administration staff, and technical staff, in order to execute the activities in our institute effectively. For these purposes, we carry out several tasks such as planning for new institutional research programs and symposiums, fundraising, supporting international students and researchers, outreach activities, providing academic advice to administration staff, and other projects directed by the dean.

1. Support for the management of institutional projects

Kiyomi Nakagawa, Yoko Udagawa

We served as a secretariat of institutional projects implemented by the Institute of Medical Science, the University of Tokyo (IMSUT) and supported their management. The projects supported are as follows:
- "Studies to Control Emerging, Re-emerging and Imported Infectious Diseases to Be Conducted in International Collaboration Sites in China" supported by Japan program of Infectious Diseases Research and Infrastructure from Japan Agency for Medical Research and Development (AMED)
- "World-leading Innovative Graduate Study Program for Life Science and Technology (WINGS-LST)" supported by the Doctoral Program for World-leading Innovative & Smart Education from Japan Society for the Promotion of Science (JSPS)

2. International Joint Usage/Research Center Program of MEXT

Junko Tsuzuku, Kaori Inoue

IMSUT was authorized by MEXT as a Joint Usage/Research Center in 2009 and began its activity in 2010. The center's main activity is to implement joint research projects, accepting applications from researchers at universities and research institutions inside and outside Japan, to organize academic gatherings such as international symposia, and meetings as well as seminars for young researchers, and to publish activity reports on our website. In addition to the above-mentioned activities, we edit documents pertaining to various investigations, and submit evaluation reports to MEXT in collaboration with the Research Promotion Team, Research Support Division, Administration Office. IMSUT was authorized as an International Joint Usage/Research Center by MEXT in November 2018 and approved to continue the center program for the next six years in line with MEXT's policy in December 2021. In this capacity, we will continue our utmost efforts to further expand this program both on domestic and international levels.

3. Data acquisition about research and educational activities of IMSUT

Kiyomi Nakagawa, Ayako Miyake

We collected and stocked data using an original format to construct a data system available any time for evaluation, submission of various reports, public relation activities, and basic data for application of external funding.

4. Others

Kiyomi Nakagawa, Ayako Miyake, Yoko Udagawa

a. Educational activities:
- Support for the call for application and selection of the Outstanding Student Publication Award of IMSUT

b. International activities:
- Support for conclusion and renewal of MOUs
- Support for delegation and management of international exchange events, "East Asia Joint Symposium on Biomedical Research" and "International Symposium of the Institute Network for Biomedical Sciences"
- Translation of documents and manuscripts
- Support for foreign researchers in English
- Support for reception of overseas visitors
- Support for management of the University of Tokyo New York Office, Inc. and its event organization
- Planning and running of get-together party for international students and foreign researchers

c. Public relations:
- Support for information update of IMSUT website
- Edition of brochures of IMSUT (Japanese and English version) and support for edition of the Annual Report

d. Support for evaluation work:
- National university corporation evaluation
- Self-review and self-evaluation of IMSUT
- External review of IMSUT

Dean's Office

Research Platform Office
学術研究基盤支援室

Chair and Senior Professor	Jun-ichiro Inoue, Ph.D.		特命教授・室長	薬学博士	井　上　純一郎
Vice chair and Professor	Mutsuhiro Takekawa, M.D., Ph.D.		教授・副室長	博士(医学)	武　川　睦　寛

"Platforms for Advanced Technologies and Research Resources" (platform.umin.jp/) was launched in fiscal year (FY) 2016 under the new framework of the Grant-in-Aid for Scientific Research on Innovative Areas by the Ministry of Education, Culture, Sports, Science and Technology (MEXT). It consists of six platforms, of which four platforms are supporting researches in life science. They are the platforms that have been developed from and strengthened the previous program "Support Programs for Three Fields in Life Sciences (Cancer, Genome and Brain Sciences)" conducted between FY 2010 and 2015. "Platforms for Advanced Technologies and Research Resources" aims to establish the academic research support platforms to efficiently support various needs of the researchers in grants-in-aid. It also aims to work in close cooperation with the relevant core bodies such as Inter-University Research Institutes and Joint Usage/ Research Centers. This office mainly plays the role of the representative secretariat of the "Committee on Promoting Collaboration in Life Sciences" that is an academic collaborative foundation and cooperates with the four platforms mentioned above. The objective is to contribute the further development of the academic research in Japan through providing the cutting-edge technologies and biological resources to the individual researchers on life science KAKENHI (Grants-in-Aid for Scientific Research). We also aim to promote cooperation among researchers across support functions and cross-disciplinary, as well as human resource development. To achieve the goal, the General Management Group was organized to facilitate a close cooperation between four platforms comprising 54 universities and 23 research institutions nationwide which provide 81 support functions. This office was established in this Institute as Dean's Office in 2016 in order to strengthen the flexible management. Further, we hold several Management Board Meetings in which 17 members participated: four platform representatives and 13 board members, to construct a cooperative system to facilitate a cross-over support functions and to provide technical support with the universities and research institutions nationwide.

Management of "Committee on Promoting Collaboration in Life Sciences" and the two platforms: Advanced Animal Model Support (AdAMS) and Cohort Study and Biospecimen Analysis (CoBiA):

Jun Saito, Tomoko Fujita, Yuko Sonoda, Mutsuhiro Takekawa and Jun-ichiro Inoue

The following activities have been performed under the management of this office in 2021.

1. Planning and organization of the budgetary allocation.
2. One-stop service for applicants through the home page.
3. Organization of the events for developing young scientists and interdisciplinary researches.
4. Holding public symposiums on the relation of life science and society.
5. Holding the explanatory meeting for possible applicants.
6. Conducting public relations activities such as posting the committee's banner on home page of various scientific meetings.
7. Creating a video to promote our activities and upload it on YouTube.
8. Facilitating cooperative networks between our platforms and other domestic or international groups that support life science researches.

Dean's Office

International Affairs Office
国際学術連携室

Professor Makoto Nakanishi, M.D., Ph.D.

教 授 医学博士 中 西 真

International Affairs Office consists of two parts: one concerned with public relations and the other with language assistance. The office is responsible for public relations activities strategy and publishes new information about a variety of scientific research of IMSUT on its official website and social media. The office also works towards increasing IMSUT's international presence by issuing press releases both in Japanese and English, holding press conferences, and editing public relations magazines of IMSUT. For its part in language assistance, the staff contributes to the creation of a favourable environment for international members of IMSUT by providing Japanese-English (or vice versa) language support including translation of useful information and official documents.

1. Publication of Press Releases on IMSUT research results

Asako Shimizu

The office issued press releases on various new findings from IMSUT, including SARS-CoV-2, Genomic medicine, intractable diseases, and distributed Japanese press releases to media institutions and science journalists strategically. The office also disseminated English press releases to the global community of science journalists through the official website, social media such as Twitter and Facebook, along with the international public relations website "Eurek Alert!".

2. Publication of the Public Relations magazine

Asako Shimizu

The office worked closely with the faculty members who belong to the public relations magazine working group and published PR magazine "PLATINUM STREET TIMES" featuring IMSUT's research achievements on the latest various virus and genome studies in June and December 2021.

3. Language Assistance

Kazuyo Ohara

In 2021, during the COVID-19 pandemic, the International Affairs Office worked closely with the administrative staff to provide international members of IMSUT with the Dean's messages, official announcements, and relevant information in English where necessary. Our language support also contributed to the success of the 27th East Asia Joint Symposium, which took place entirely online. The office will continue to promote the internationalization at IMSUT and a favourable environment for international members of IMSUT by offering tailored language services.

Dean's Office

BioBank Japan
バイオバンク・ジャパン

Professor	Yuji Yamanashi, Ph.D.	教　授	理学博士　山　梨　裕　司
Project Professor	Takayuki Morisaki, M.D., Ph.D.	特任教授	医学博士　森　崎　隆　幸
Professor	Koichi Matsuda, M.D., Ph.D.	連携教授	博士（医学）（大学院新領域創成科学研究科）　松　田　浩　一
Professor	Yoichiro Kamatani, M.D., Ph.D.	連携教授	博士（医学）（大学院新領域創成科学研究科）　鎌　谷　洋一郎

In 2003, BioBank Japan (BBJ) started establishing one of the world's largest disease biobanks, creating a foundation for genomic and clinical research. From a total of 267,000 patients representing 440,000 cases of 51 primarily multifactorial diseases, BBJ has collected DNA, serum, medical records. BBJ is promoting the utilization of the registered samples and data acquired over the years, resulting in important research findings contributing to the realization of genomic medicine.

Publication

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Schraut, Christopher Scott, Saleh Shekari1, Amruta Shrikhande3, Albert V Smith, Blair H Smith, Jennifer A Smith, Rossella Sorice, Melissa C Southey, Tim D Spector, John J Spinelli, Meir Stampfer, Doris Stöckl, Joyce BJ van Meurs, Konstantin Strauch, Unnur Styrkarsdottir, Anthony J Swerdlow, Toshiko Tanaka, Lauren R Teras, Alexander Teumer, Unnur Þorsteinsdottir, Nicholas J Timpson, Daniela Toniolo, Michela Traglia, Melissa A Troester, Thérèse Truong, Jessica Tyrrell1, André G Uitterlinden, Sheila Ulivi, Celine M Vachon, Veronique Vitart, Uwe Völker, Peter Vollenweider, Henry Völzke, Qin Wang, Nicholas J Wareham, Clarice R Weinberg, David R Weir, Amber N Wilcox, Ko Willems van Dijk, Gonneke Willemsen, James F Wilson, Bruce HR Wolffenbuttel, Alicja Wolk, Andrew R Wood1, Wei Zhao, Marek Zygmunt, Biobank-based Integrative Omics Study (BIOS) Consortium, eQTLGen Consortium, The Biobank Japan Project, China Kadoorie Biobank Collaborative Group, kConFab Investigators, The LifeLines Cohort Study, The InterAct consortium, 23andMe Research Team, Zhengming Chen, Liming Li, Lude Franke, Stephen Burgess, Patrick Deelen, Tune H Pers, Marie Louise Grøndahl, Claus Yding Andersen, Anna Pujol, Andres J Lopez-Contreras, Jeremy A Daniel, Kari Stefansson, Jenny Chang-Claude, Yvonne T van der Schouw, Kathyrn L Lunetta, Daniel I Chasman, Douglas F Easton, Jenny A Visser, Susan E Ozanne, Satoshi H Namekawa, Petr Solc, Joanne M Murabito, Ken K Ong, Eva R Hoffmann, Anna Murray, Ignasi Roig and John RB Perry Genetic insights into the biological mechanisms governing human ovarian ageing. Nature 2021 Aug;596(7872):393-397.

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Yves Akoli Koudou, Esther M John, Eli Marie Grindedal, Lovise Maehle, Kay-Tee Khaw, Sue A Ingles, Mariana C Stern, Ana Vega, Antonio Gómez-Caamaño, Laura Fachal, Barry S Rosenstein, Sarah L Kerns, Harry Ostrer, Manuel R Teixeira, Paula Paulo, Andreia Brandão, Stephen Watya, Alexander Lubwama, Jeannette T Bensen, Elizabeth T H Fontham, James Mohler, Jack A Taylor, Manolis Kogevinas, Javier Llorca, Gemma Castaño-Vinyals, Lisa Cannon-Albright, Craig C Teerlink, Chad D Huff, Sara S Strom, Luc Multigner, Pascal Blanchet, Laurent Brureau, Radka Kaneva, Chavdar Slavov, Vanio Mitev, Robin J Leach, Brandi Weaver, Hermann Brenner, Katarina Cuk, Bernd Holleczek, Kai-Uwe Saum, Eric A Klein, Ann W Hsing, Rick A Kittles, Adam B Murphy, Christopher J Logothetis, Jeri Kim, Susan L Neuhausen, Linda Steele, Yuan Chun Ding, William B Isaacs, Barbara Nemesure, Anselm J M Hennis, John Carpten, Hardev Pandha, Agnieszka Michael, Kim De Ruyck, Gert De Meerleer, Piet Ost, Jianfeng Xu, Azad Razack, Jasmine Lim, Soo-Hwang Teo, Lisa F Newcomb, Daniel W Lin, Jay H Fowke, Christine Neslund-Dudas, Benjamin A Rybicki, Marija Gamulin, Davor Lessel, Tomislav Kulis, Nawaid Usmani, Sandeep Singhal, Matthew Parliament, Frank Claessens, Steven Joniau, Thomas Van den Broeck, Manuela Gago-Dominguez, Jose Esteban Castelao, Maria Elena Martinez, Samantha Larkin, Paul A Townsend, Claire Aukim-Hastie, William S Bush, Melinda C Aldrich, Dana C Crawford, Shiv Srivastava, Jennifer C Cullen, Gyorgy Petrovics, Graham Casey, Monique J Roobol, Guido Jenster, Ron H N van Schaik, Jennifer J Hu, Maureen Sanderson, Rohit Varma, Roberta McKean-Cowdin, Mina Torres, Nicholas Mancuso, Sonja I Berndt, Stephen K Van Den Eeden, Douglas F Easton, Stephen J Chanock, Michael B Cook, Fredrik Wiklund, Hidewaki Nakagawa, John S Witte, Rosalind A Eeles, Zsofia Kote-Jarai, Christopher A Haiman Trans-ancestry genome-wide association meta-analysis of prostate cancer identifies new susceptibility loci and informs genetic risk prediction Nature Genetics 2021 Jan;53(1):65-75

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SCIENTIFIC MEETINGS & SEMINARS

48th IMSUT Founding Commemorative Symposium Advance in gene therapies and genome editing tools

本研究所では伝染病研究所から医科学研究所への改組を記念して創立記念シンポジウムを毎年開催している。

本年は「遺伝子治療とゲノム編集技術の進歩」というテーマで講演をお願いした。

日　時：令和 3 年 5 月 28 日（金）　13：00 ～ 17：00
会　場：Zoom によるオンライン開催

Takashi Okada（Division of Molecular and Medical Genetics, Center for Gene and Cell Therapy , IMSUT）
Innovation in AAV vector technologies toward gene and cell-based therapies to treat muscular dystrophy

Keiya Ozawa（Division of Immuno-Gene & Cell Therapy (Takara Bio), Jichi Medical University）
Gene therapy comes of age

Tomoji Mashimo（Division of Animal Genetics, Laboratory Animal Research Center, Institute of Medical Science, IMSUT）
A novel genome editing tool CRISPR-Cas3

Osamu Nureki（Department of Biological Sciences, Graduate School of Science, The University of Tokyo）
Development of novel genome-editing tools towards gene therapy

Takashi Yamamoto（Division of Integrated Sciences for Life, Graduate School of Integrated Sciences for Life, Hiroshima University）
Genome editing in various organisms using Platinum TALEN and CRISPR-Cas9

学友会セミナー

（令和３年１月～令和３年 12 月）

２月８日　演題：　翻訳の伸長異常を感知する品質管理機構
　　演者：　松尾　芳隆
２月８日　演題：　内在性レトロウイルスの駆動する生命現象および進化
　　演者：　伊東　潤平
２月22日　演題：　異なる上皮の接点の発生・維持機構の解明と組織特異的にシグナルパスウェイの活性化を制御可能な新規マウスモデルの作製
　　演者：　三小田　直
３月９日　演題：　データサイエンスとスーパーコンピューティングによる全ゲノム臨床シークエンスの実現
　　演者：　片山　琴絵
４月１日　演題：　CNOT 複合体を介した mRNA 発現制御の組織恒常性における役割
　　演者：　鈴木　　亨
５月６日　演題：　肝臓における上皮細胞供給システムの解明に基づく Ex vivo 組織再構築法の創出
　　演者：　谷水　直樹
５月13日　演題：　遺伝子改変マウスモデル作出技術の高度化とその応用について
　　演者：　小沢　　学
５月28日　演題：　造血器腫瘍における PRC2 の役割
　　演者：　青山　和正
５月31日　演題：　Bioinformatic Methods for Studying Adenosine-to-Inosine RNA Editing
　　演者：　Tyler Weirick
６月23日　演題：　ヒト表皮幹細胞の動態解析と再生医療応用
　　演者：　難波　大輔
７月12日　演題：　次世代医療技術創出のための革新的診断技術応用基盤
　　演者：　安井　　寛
７月28日　演題：　インフルエンザウイルスは空気感染するのか？～なぜ、H5N1 ウイルスはパンデミックを起こさないのか～
　　演者：　岩附　研子
９月８日　演題：　ヒト癌原性タンパク質 5MP1 による一般的及びリピート依存性 non-AUG 翻訳制御の共通の分子機構
　　演者：　浅野　　桂
９月13日　演題：　Lysosomal amino acid transporters integrate inflammatory and metabolic signaling in macrophages
　　演者：　反町 典子
９月14日　演題：　Integration of single-cell transcriptome replicates using linear estimation and fuzzy logic

演者：　Martin de Jesus Loza López

10月15日　演題：　白血病治療法確立に向けた基礎と臨床

演者：　小沼　貴晶

11月 8 日　演題：　造血器悪性腫瘍を対象とした腫瘍特異的治療法の開発

演者：　二見　宗孔

11月30日　演題：　オルガノイドと組織 1 細胞解析の統合によるがん治療抵抗性ニッチの理解

演者：　岡本　康司

12月17日　演題：　感染記憶による Elf1 活性化を介した骨髄異形成症候群の発症機序の解析

演者：　横溝　貴子

12月28日　演題：　HIV 感染者における腸内細菌叢の変化と慢性炎症

演者：　石坂　彩

EDUCATION

大学院セミナー

医科学研究所では、毎年テーマを決めて大学院生を対象としたセミナーを開いている。各々の年の決定されたテーマに関していろいろな視点から最先端の研究を展開しておられる方々に講師をお願いし、現在どのような研究が進められていて、どこまで明らかにされているかが幅広く理解できるように計画が立てられている。2021 年には、「感染症克服について」というテーマの下で次のようなセミナーが行われた。

感染症克服について

	月　日	講師名		演　　題
1	4 月 5 日	今井　由美子	国立研究開発法人医薬基盤・健康・栄養研究所 プロジェクトリーダー	ウイルス感染症の重症化メカニズム
2	4 月 12 日	川口　　寧	東京大学医科学研究所 ウイルス病態制御分野 教授	ヘルペスウイルスの増殖・病態発現機構
3	4 月 19 日	橋口　隆生	京都大学ウイルス・再生医科学研究所 教授	RNA ウイルスの細胞侵入機構と侵入阻害による感染制御機構の研究
4	4 月 26 日	田中　靖人	熊本大学大学院生命科学研究部 消化器内科学 教授	ウイルス性肝炎撲滅を目指して
5	5 月 10 日	中川　一路	京都大学大学院医学研究科 微生物感染症学分野 教授	菌種特異的な増殖阻害剤の開発：構造解析を基礎としたモダリティ分子による細菌感染症の制御
6	5 月 17 日	渡辺　登喜子	大阪大学微生物病研究所 感染機構研究部門分子ウイルス分野 教授	新興感染症の制圧を目指して
7	5 月 24 日	田中　幹人	早稲田大学政治経済学術院 准教授	リスクコミュニケーションの現在と課題
8	10 月 4 日	高橋　宜聖	国立感染症研究所免疫部 部長	感染症対策に資する免疫研究
9	10 月 18 日	松岡　雅雄	熊本大学生命科学研究部 血液・膠原病・感染症内科学講座 教授	ヒト T 細胞白血病ウイルス I 型の病原性発現機構
10	10 月 25 日	安田　二朗	長崎大学感染症共同研究拠点 教授	新興ウイルス感染症と BSL- 4 施設
11	11 月 1 日	畠山　昌則	東京大学医学系研究科 微生物学分野 教授	細菌感染発がんのロジック
12	11 月 8 日	四柳　　宏	東京大学医科学研究所 感染症分野 教授	HIV 感染症の克服をめざして
13	11 月 15 日	山﨑　　晶	大阪大学微生物病研究所 分子免疫制御分野 教授	T cell responses against SARS-Co V2

学術フロンティア講義

医科学研究所では、教養学部前期課程の学生を対象に、「医科学研究最前線」として、平成 27 年度から学術フロンティア講義を開講している。研究所を構成する６つの基幹部門・施設から選出された講師が、それぞれの研究分野の最新の動向をわかりやすく講義した。

日　時：令和３年 12 月 11 日（土）９：15 ～ 16：40
　　令和３年 12 月 12 日（日）９：30 ～ 16：40
場　所：Zoom によるオンラインでの開講

教員および題目

12 月 11 日（土）

講　師　名		題　　目
中西　　真	癌・細胞増殖部門 癌防御シグナル分野	医科研紹介
井元　清哉	ヒトゲノム解析センター 健康医療インテリジェンス分野	ゲノム研究の新次元
稲田　利文	基礎医科学部門 RNA 制御学分野	リボソーム修飾による動態制御と、その破綻による疾患の分子機構
福井　竜太郎	感染・免疫部門 感染遺伝学分野	自然免疫系を標的とした自己免疫疾患の制御
加藤　哲久	感染・免疫部門 ウイルス病態制御分野	ヘルペスウイルスの病態発現機構

12 月 12 日（日）

講　師　名		題　　目
北村　俊雄	先端医療研究センター 細胞療法分野	クローン性造血と種々の疾患の関係／エピジェネティクって何？
岡田　尚巳	遺伝子・細胞治療センター 分子遺伝医学分野	遺伝子治療の開発動向と課題
西村　栄美	癌・細胞増殖部門 老化再生生物学分野	組織幹細胞から迫る臓器の老化の仕組み
小沢　　学	システム疾患モデル研究センター 生殖システム研究分野	ゲノム編集技術を駆使して生殖細胞の振る舞いを理解する

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